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(54) Title: KAHALALIDE COMPOUNDS

(57) Abstract: A process is provided for preparing kahalalide F and which leads to other kahalalide mimic compounds having useful biological activity.

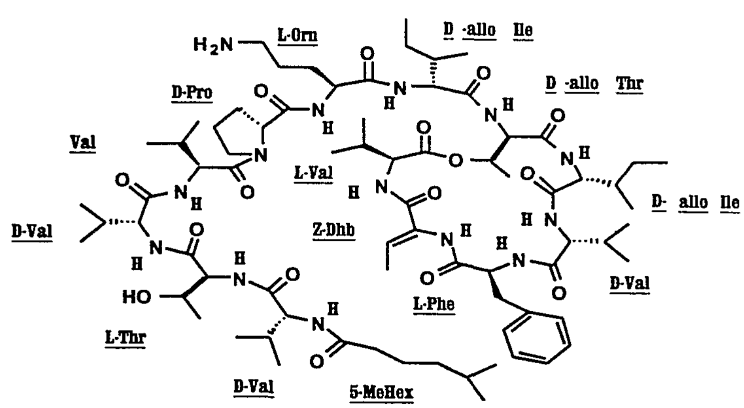
KAHALALIDE COMPOUNDS

The present invention relates to kahalalide compounds, and in particular to kahalalide F and related compounds, as well as a synthetic route for such compounds.

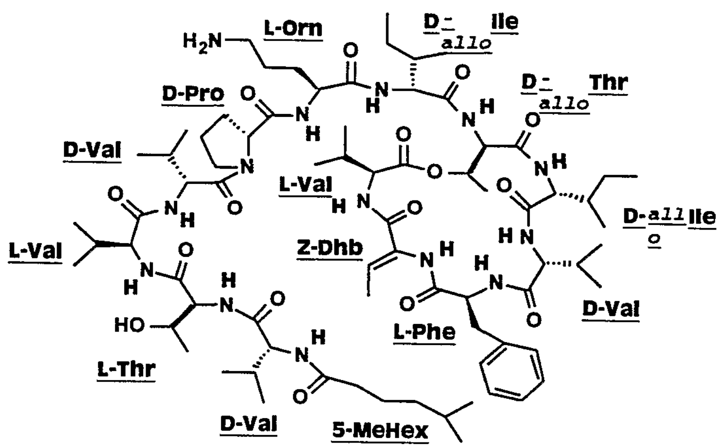
BACKGROUND OF THE INVENTION

Kahalalide F is a bioactive cyclic depsipeptide isolated from the sarcoglossan mollusc *Elysia rufescens* and its diet, the green alga *Bryopsis* sp. Kahalalide F was first isolated by Hamann and Scheuer, see Hamann, M.T.; Scheuer, P.J. J. Am. Chem. Soc. 1993, 115, 5825-5826. In this publication, the absolute stereochemistry of individual valines (3 D-Val and 2 L-Val) and of threonines (L-Thr and D-allo-Thr) was not determined. In a later publication, Scheuer *et al.* (Goetz, G.; Yoshida, W.Y.; Scheuer, P.J. Tetrahedron 1999, 55, 7739-7746) assigned a position in the molecule for the 5 Val and the 2 Thr.

Thus, the structure for Kahalalide F according to Scheuer *et al.* was:



In the meantime, Prof. Reinhart also assigned the individual position of the 5-Val and the 2-Thr (K.L. Rinehart, personal communication). While for the 2 Thr and 3 Val, his assignation concords with that of Prof. Scheuer, for the last 2 Val there is a discrepancy. Thus, in the Rinehart assignment, the two consecutive Val in the side chain are switched and the structure is of the formula (I):



Formula (I)

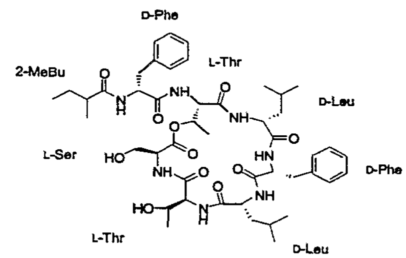
It is now accepted, and demonstrated again in this text, that the correct formula for kahalalide F is the formula (I).

The structure is complex, comprising six amino acids as a cyclic part, and an exocyclic chain of seven amino acids with a terminal acyl group. Kahalalide F (I) is an exceedingly potent and rare marine-derived antitumour agent, though the absence of adequate quantities has slowed plans for clinical trials.

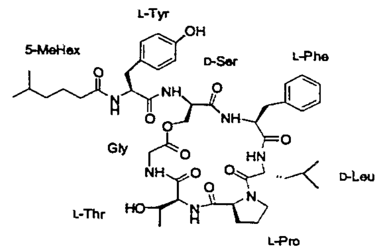
Other kahalalide compounds are known, and for example we refer to Hamann, M.T., et al., *J. Org. Chem.*, 1996, "Kahalalides: Bioactive Peptides from Marine Mollusk *Elysia rufescens* and its Algal Diet *Bryopsis* sp.", vol. 61, pp. 6594-6660. As well as kahalalide F, this article gives structures for

kahalalides A to E. Kahalalide K was reported in J Nat. Prod. 1999, 62, 1169, and kahalalide O was reported in J Nat Prod 2000, 63, 152.

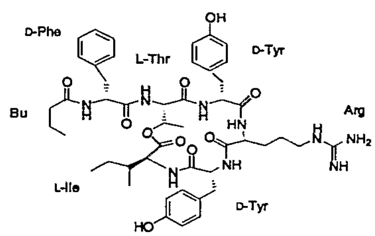
The structures for these further kahalalide compounds are shown in the following formulae:



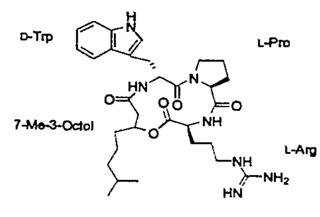
Kahalalide A (1)



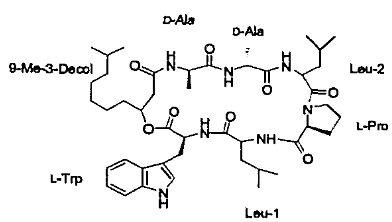
Kahalalide B (2)



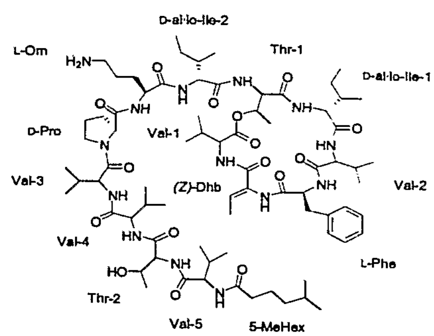
Kahalalide C (3)



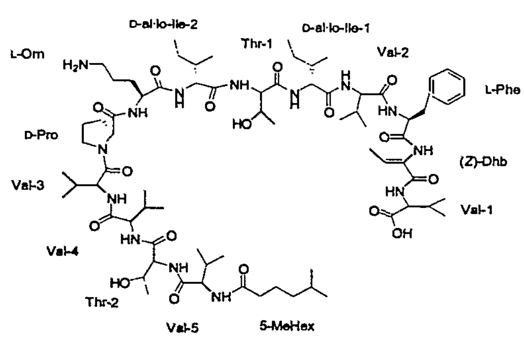
Kahalalide D (4)



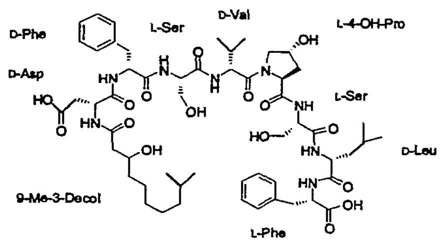
Kahalalide E (5)



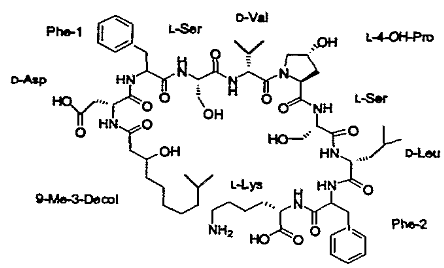
Kahalalide F (6)



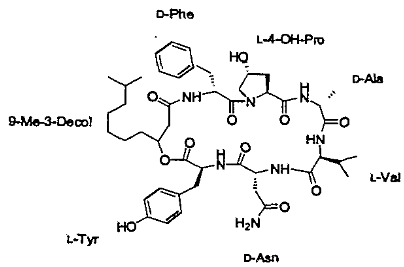
Kahalalide G (7)



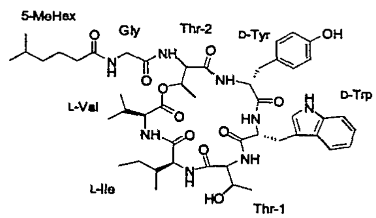
Kahalalide H (8)



Kahalalide J (9)



Kahalalide K (10)



Kahalalide O (11)

The cyclic kahalalides have a ring of amino acids, and a side chain which terminates in an acyl group.

Kahalalides have a range of biological activities, notably antitumour and antituberculosis activity.

SUMMARY OF THE INVENTION

The present invention provides mimics of natural kahalalides. The compounds may differ in one or more amino acids, and one or more components of the acyl side chain.

Suitably the mimics of this invention have at least one of the following features to differentiate from a parent naturally occurring kahalalide:

1 to 7, especially 1 to 3, more especially 1 or 2, most especially 1, amino acid which is not the same as an amino acid the parent compound;

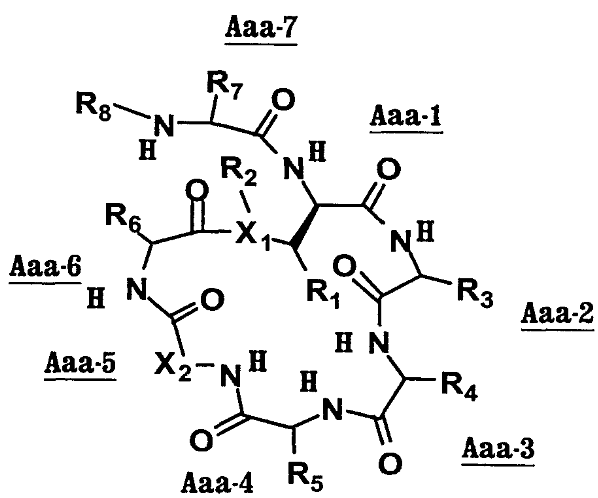
1 to 10, especially 1 to 6, more especially 1 to 3, most especially 1 or 2, additional methylene groups in the side chain acyl group of the parent compound;

1 to 10, especially 1 to 6, more especially 1 to 3, most especially 1 or 2, methylene groups omitted from the side chain acyl group of the parent compound;

1 to 6, especially 1 to 3, more especially 1 or 3, substituents added to or omitted from the side chain acyl group of the parent compound.

For cyclic kahalalides, the amino acid addition or omission can be in the cyclic ring or in the side chain.

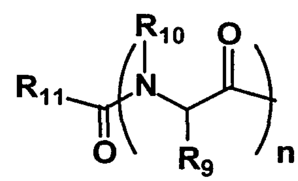
Examples of this invention are compounds related to kahalalide F, and having the formula:



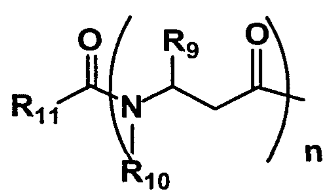
Formula II

wherein Aaa_1, Aaa_2, Aaa_3, Aaa_4, Aaa_6, and Aaa_7 are independently α-amino acids of L or D configuration, if applies; wherein R_1, R_2, R_3, R_4, R_5, R_6, R_7 are each independently H or an organic group selected from the group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto group, an amino

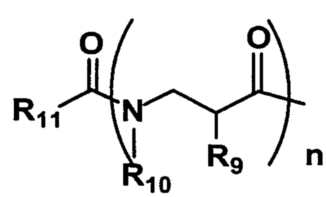
group, a guanidino group, a halogen group; wherein X_1 is independently O, S, or N; wherein R_2 is, if applies, independently H or an organic group selected from the group consisting of an alkyl group and an aralkyl group; wherein Aaa5 is independently an amino acid of L or D configuration, if applies; wherein X_2 is independently an organic group selected from the group consisting of an alkenyl, an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto independently H or an organic group selected from the group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a mercapto group, an amino group, a guanidino group, a halogen group; wherein R_8 is independently of the following formulae III, IV, or V:



Formula III



Formula IV



Formula V

wherein R_9, R_{10}, and R_{11} are each independently H or an organic group selected from the group consisting of an alkyl group, an aryl group, an aralkyl group, and their substituted derivatives with an hydroxy group, a

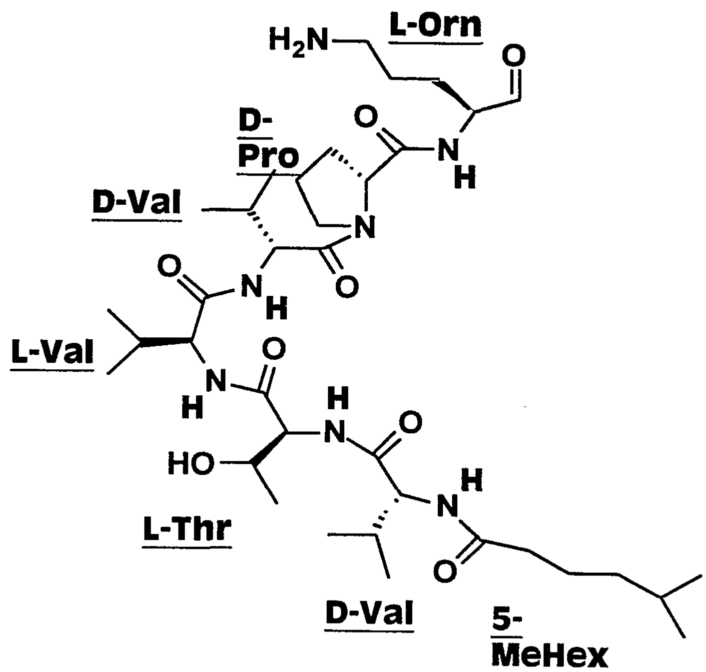
mercapto group, an amino group, a guanidino group, a carboxyl group, a carboxamido group, a halogen group; R_9 and R_{10} can form part of the same cycle; R_9 can confer S or R configuration, if applies, to the carbon attached to; and n is 0 to 6. The definitions of the amino acids can also be varied to allow for proline and analogous amino acids including hydroxyproline. The formulae (III), (IV) and (V) can be intermixed to give a side chain made up of repeat units in more than one of these formulae.

In a modification, one or more of the ring amino acids Aaa-6 and Aaa-5 of the hexaamino acid cycle is omitted or an amino acid Aaa-7 is added between Aaa-6 and Aaa-1, in order to arrive at rings having four, five or seven ring amino acids. Six ring amino acids is preferred.

The present invention is further directed to a synthetic process for the formation of Kahalalide F compounds and related structures.

PREFERRED EMBODIMENTS

Preferred compounds are related to Kahalalide F (I), wherein in the formula (II), Aaa-1 is D-*allo*-Thr (X_1 = O, R_1 = CH_3), Aaa-2 is D-*allo*-Ile (R_3 = 1-methylpropyl), Aaa-3 is D-Val (R_4 = isopropyl), Aaa-4 is Phe (R_5 = benzyl), Aaa-5 is *Z*-Dhb (X_2 = C=CHCH$_3$ with *Z* configuration), Aaa-6 is L-Val (R_6 = isopropyl), Aaa-7 is D-*allo*-Ile (R_7 = 1-methylpropyl), and R_8 contains a hexapeptide derivative of the following formula (Formula VI):



Formula VI

Other preferred compounds are analogously related to the other kahalalides, and have amino acids and side chains in common with such compounds. Reference is made to the known structures of the kahalalides. For preference, a compound of this invention mimics a natural kahalalide, with modification, insertion or suppression at one or more points in the molecule.

Especially preferred are compounds which have the same amino acids as in a natural cyclic kahalalide compound, but differ in the side chain, especially in the acyl part. Such differences can include more or fewer methylene groups, typically at most a change of two methylene groups, and more or less substituents, notably the addition of halogen such as fluorine substituents.

As used herein, the term "organic group" means a hydrocarbon group that is classified as an aliphatic group, cyclic group, or combination of

aliphatic and cyclic groups (*e.g.,* aralkyl groups). In the context of the present invention, the term "aliphatic group" means a saturated or unsaturated linear or branched hydrocarbon. This term is used to encompass alkyl, alkenyl, and alkynyl groups, for example.

The term "alkyl group" means a saturated linear or branched hydrocarbon group including, for example, methyl, ethyl, isopropyl, isobutyl, *t*-butyl, heptyl, dodecyl, octadecyl, amyl, 2-ethylhexyl, 2-methylbutyl, 5-methylhexyl, 9-methyl-3-decyl, and the like, particularly alkyl groups which have a single branched methyl group. Suitably the alkyl group is long and has 1 to 20 carbon atoms, more typically 1 to 15 or 1 to 10 carbon atoms, or can be short and has 1 to 6 or 1 to 3 carbon atoms. The group R_{11} is suited to the long carbon chains, and in general all the other alkyl groups are preferably short.

The term "alkenyl group" means an unsaturated, linear or branched hydrocarbon group with one or more carbon-carbon double bonds, such as a vinyl group. Suitably the alkenyl group has 2 to 8 or 2 to 4 carbon atoms.

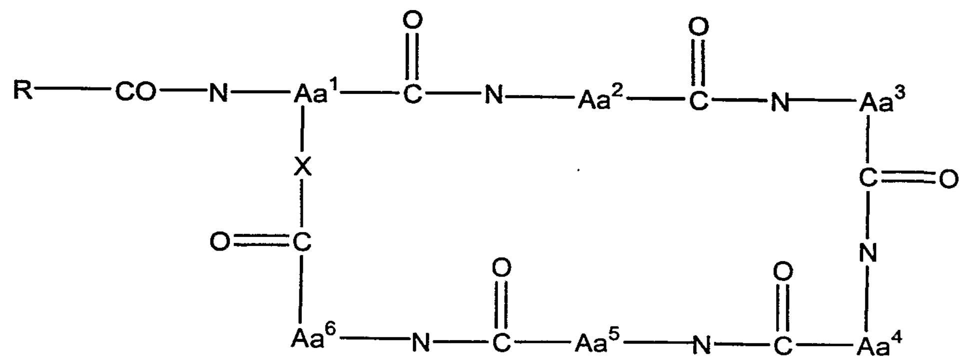
The term "alkynyl group" means an unsaturated, linear or branched hydrocarbon group with one or more carbon-carbon triple bonds. Suitably the alkynyl group has 2 to 8 or 2 to 4 carbon atoms.

The term "cyclic group" means a closed ring hydrocarbon group that is classified as an alicyclic group, aromatic group, or heterocyclic group. The term "alicyclic group" means a cyclic hydrocarbon group having properties resembling those of aliphatic groups, and is suitably a mono- or bi-cycle with 4 to 10 carbon atoms. The term "aromatic group" or "aryl group" means a mono- or polycyclic aromatic hydrocarbon group, suitably a mono- or bi-cycle with 5 to 10 carbon atoms. The term "heterocyclic group" means a closed ring hydrocarbon in which one or more of the atoms in the ring is an

element other than carbon (*e.g.*, 1 to 3 of one or more of nitrogen, oxygen, sulfur, etc.), suitably a mono- or bi-cycle with 4 to 10 ring atoms.

As is well understood in this technical area, a large degree of substitution is not only tolerated, but is often advisable. Substitution is anticipated on the compounds of the present invention. As a means of simplifying the discussion and recitation of certain terminology used throughout this application, the terms "group" and "moiety" are used to differentiate between chemical species that allow for substitution or that may be substituted and those that do not allow or may not be so substituted. Thus, when the term "group" is used to describe a chemical substituent, the described chemical material includes the unsubstituted group and that group with O, N, or S atoms, for example, in the chain as well as carbonyl group or other conventional substitution. Where the term "moiety" is used to describe a chemical compound or substituent, only a unsubstituted chemical material is intended to be included. For example, the phrase "alkyl group" is intended to include not only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, isobutyl, and the like, but also alkyl substituents bearing further substituents known in the art, such as hydroxy, alkoxy, amino, carboxyl, carboxamido, halogen atoms, cyano, nitro, alkylsulfonyl, etc. Thus, "alkyl group" includes ether groups, haloalkyls, alcohols, thiols, carboxyl, amines, hydroxyalkyls, sulfoalkyls, etc. Haloalkyl groups are currently preferred. On the other hand, the phrase "alkyl moiety" is limited to the inclusion of only pure open chain saturated hydrocarbon alkyl substituents, such as methyl, ethyl, propyl, isobutyl, and the like.

In a particularly preferred embodiment of this invention, we provide a compound of the formula (A):



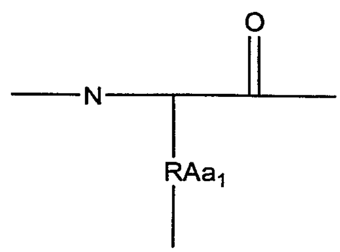
where each of Aa^1 to Aa^6 with the adjacent respective –N- and –CO- represents an α or β amino acid, preferably an α amino acid, X is a linking group of Aa^1, preferably selected from amino, hydroxy or thiol, more preferably hydroxy, and R is a group optionally including one or more α or β amino acids, preferably α amino acids. In a variation, -CO-Aa^6-N- may be absent, possibly along with –CO-Aa^5-N- also being absent, or alternatively a group –CO-Aa^7-N- may be interposed between Aa^6 and Aa^1.

The amino acids can be of L or D configuration. Preferably the configuration is the same as the corresponding amino acid in a kahalalide comound especially kahalalide F. One or more of Aa^1 to Aa^7 preferably corresponds to an amino acid at the same position in a natural kahalalide, or a congener thereof. In a particularly preferred embodiment, each amino acid is the same as in kahalalide F. Thus, for preference, Aa^1 is D-*allo*-Thr, Aa^2 is D-*allo*-Ile, Aa^3 is D-Val, Aa^4 is Phe, Aa^5 is *Z*-Dhb, Aa^6 is L-Val.

Where congeners are employed, suitable congeners have substitute amino acids where the substitution is made having regard to the structure of the respective amino acid in the natural kahalalide such as kahalalide F. Substitutes for threonine include serine and other homologues as well as cysteine and homologues. Substitutes for isoleucine include other amino acids with nonpolar groups, including alanine, valine, leucine, and proline, as well as analogues and homologues. In this respect, the showing of the

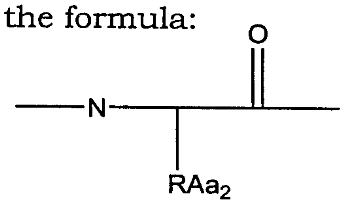
nitrogen atoms –N- in the formula (A) does not exclude the possibility of ring formation such as occurs with proline. Substitutes for valine include isoleucine and the other amino acids with nonpolar groups, including analogues and homologues. Substitutes for phenylalanine include tryptophan and tyrosine. Substitutes for Dhb include other amino acids with such an alkenylidene substituent, including amino acids with C_3 or higher sidechains, as well as analogues and homologues.

Preferably Aa^1 is of the formula:



where Raa_1 is a sidechain carrying the linking X. Examples of sidechains include alkyl, aryl and aralkyl, especially alkyl, more typically alkyl of 1 to 4, such as 2 carbon atoms. Branched alkyl is preferred. The linking group X is suitably oxygen, with Aa_1 being preferably derived from threonine, but sulphur, amino (unsubstituted or substituted with alkyl, aryl or aralkyl), and other linkers can be employed. Suitably the group X is the only polar group in Aa^1. Aa^1 can be racemic, L or D, but is preferably in the D configuration.

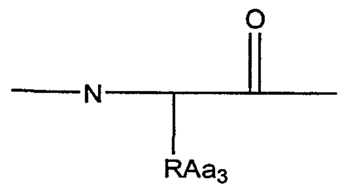
Preferably Aa^2 is of the formula:



where Raa_2 is a sidechain such as alkyl, aryl and aralkyl, especially alkyl, more typically alkyl of 1 to 5, such as 3 carbon atoms. Branched alkyl is

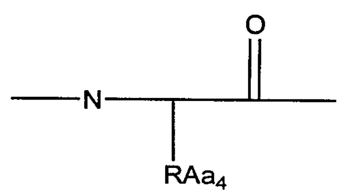
preferred, as in isoleucine. Suitably there are no polar substituents in Aa^2. Aa^2 can be racemic, L or D, but is preferably in the D configuration.

Preferably Aa^3 is of the formula:



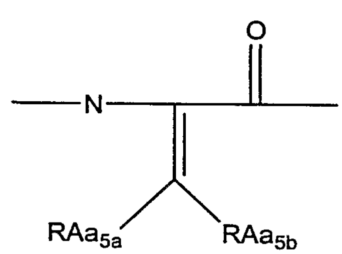
where Raa_3 is a sidechain such as alkyl, aryl and aralkyl, especially alkyl, more typically alkyl of 1 to 5 such as 3 carbon atoms. Branched alkyl is preferred, as in valine. Suitably there are no polar substituents in Aa^3. Aa^3 can be racemic, L or D, but is preferably in the D configuration.

Preferably Aa^4 is of the formula:



where Raa_4 is a sidechain such as alkyl, aryl and aralkyl, especially aralkyl, more typically aralkyl of 7 to 10 such as 7 carbon atoms. Benzyl is preferred, as in phenylalanine. Suitably there are no polar substituents in Aa^4. Aa^4 can be racemic, L or D, but is preferably in the L configuration.

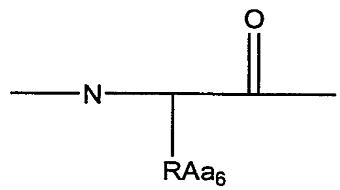
Preferably Aa^5 is of the formula:



where Raa_{5a} and Raa_{5b} is each chosen from hydrogen, or a sidechain such as alkyl, aryl and aralkyl, especially alkyl, more typically alkyl of 1 to 4 such as

1 carbon atom. Preferably Raa_{5a} is hydrogen, giving a Z configuration as in the preferred *a,b*-didehydro-a-aminobutyric acid. Suitably there are no polar substituents in Aa^5. Aa^5 can be racemic, L or D, but is preferably in the L configuration.

Preferably Aa^6 is of the formula:



where Raa_6 is a sidechain such as alkyl, aryl and aralkyl, especially alkyl, more typically alkyl of 1 to 5 such as 3 carbon atoms. Branched alkyl is preferred, as in valine. Suitably there are no polar substituents in Aa^6. Aa^6 can be racemic, L or D, but is preferably in the L configuration.

The amino acids Aa^1 to Aa^7 can be as defined for the groups Aaa-1 to Aaa-7 of formula (II).

Preferably the side chain R comprises 5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-*allo*-Ile, or a congener thereof, including analogues and homologues. Examples of congeners include those of the general formula $(R^t)_m$-(amino $acid)_n$-, with m + n non-zero, where R^t represents a terminal group, suitably an alkyl, alkenyl, alkynyl, aryl, aralkyl, heteroaryl, heteroalkyl, alicyclic or other terminator, and -(amino $acid)_n$- repersents an optional chain of amino acids.

A terminal alkyl group suitably has 1 to 12 carbon atoms, more typically 4 to 10 carbon atoms, and may be substituted and is preferably branched. Examples include butyl, pentyl, hexyl, heptyl, octyl, and other alkyl groups bearing 1 or more methyl or ethyl or longer side groups preferably of 1 to 6 carbon atoms, especially 5-methylhexyl and other alkyl

groups with branching distal to the point of attachment to the rest of the molecule. Suitable substituents include halogen, hydroxy, alkoxy, amino, carboxyl, carboxamido, cyano, nitro, alkylsulfonyl, alkoxy, alkoxyalkyl, arylalkylaryl, heterocyclic, alicyclic, aryl, aralkyl and other groups mentioned herein. As appropriate, such substituents may bear further substituents, as for example a tolyl group. Halogen substituents are currently most preferred, especially 1 or more, such as 1 to 3, flourine atoms. Other terminal groups can be selected according to the guidance given in this text, and include alicyclic, aralkyl, aryl, heterocyclic or other groups, possibly with 1 or more, especially 1 to 3, susbtituents.

The side chain R-CO-N- can have hydrogen or a substituent on the nitrogen, such as alkyl, aryl or aralkyl group.

Suitable side chain congeners employ substitute amino acids chosen in accordnace with the principles given before, with alternatives to isoleucine, valine and threonine being selected as for these amino acids in the cyclic part of the molecule, and alternatives to ornithine inclluding lysine, histidine, arginine; alternatives to proline including alanine and other nonpolar amino acids such as glycine, isoleucine and the others previously mentioned. Typically the chain R has 0 to 10, more preferably 4 to 8, such as 5, 6 or 7, especially 7, amino acid residues. Where there are more than seven amino acids, then the extra amino acids are preefrably naturally occuring amino acids, particularly those mentioned herein.

Racemic or L or D amino acids can be used. Preferably where amino acids are present in the side chain R, then one or more of them is in the configuration given for the naturally occuring sidechain. Reading from the cylic hexapeptide, the amino acid residues are in the configuration DLDDLLD. The terminal alkyl group suitably has 1 to 12 carbon atoms, more typically 4 to 10 carbon atoms, and may be substituted and is

preferably branched. Suitable substituents include halogen, hydroxy, alkoxy, amino, carboxyl, carboxamido, cyano, nitro, alkylsulfonyl and other groups mentioned herein. Halogen substituents are current most preferred.

More generally, the side chain R-CO-N- can be as defined for the side chain R_8-NH-CHR$_7$-CO-NH- in the compounds of formula (II), including compounds with mixtures of amino acids defined within formulae (III), (IV) and (V).

Although the preferrred features have been described by reference to kahalalide F, other preferred compounds of this invention are provided by the application of the same principles to the design of mimics of other kahalalides. Thus, the amino acids and side chains can be changed, preferably while retaining the hydrophobic-hydrophilic balance between polar and nonpolar groupings.

Compounds of this invention have useful biological activity, including an antitumour activity.

Thus, the present invention provides a method of treating any mammal, notably a human, affected by cancer which comprises administering to the affected individual a therapeutically effective amount of a compound of the invention, or a pharmaceutical composition thereof.

The present invention also relates to pharmaceutical preparations, which contain as active ingredient a compound or compounds of the invention, as well as the processes for their preparation.

Examples of pharmaceutical compositions include any solid (tablets, pills, capsules, granules, etc.) or liquid (solutions, suspensions or emulsions) with suitable composition or oral, topical or parenteral

administration, and they may contain the pure compound or in combination with any carrier or other pharmacologically active compounds. These compositions may need to be sterile when administered parenterally.

Administration of the compounds or compositions of the present invention may be by any suitable method, such as intravenous infusion, oral preparations, intraperitoneal and intravenous administration. We prefer that infusion times of up to 24 hours are used, more preferably 2-12 hours, with 2-6 hours most preferred. Short infusion times which allow treatment to be carried out without an overnight stay in hospital are especially desirable. However, infusion may be 12 to 24 hours or even longer if required. Infusion may be carried out at suitable intervals of say 2 to 4 weeks. Pharmaceutical compositions containing compounds of the invention may be delivered by liposome or nanosphere encapsulation, in sustained release formulations or by other standard delivery means.

The correct dosage of the compounds will vary according to the particular formulation, the mode of application, and the particular situs, host and tumour being treated. Other factors like age, body weight, sex, diet, time of administration, rate of excretion, condition of the host, drug combinations, reaction sensitivities and severity of the disease shall be taken into account. Administration can be carried out continuously or periodically within the maximum tolerated dose.

The compounds and compositions of this invention may be used with other drugs to provide a combination therapy. The other drugs may form part of the same composition, or be provided as a separate composition for administration at the same time or a different time. The identity of the other drug is not particularly limited, and suitable candidates include:

a) drugs with antimitotic effects, especially those which target cytoskeletal elements, including microtubule modulators such as taxane

drugs (such as taxol, paclitaxel, taxotere, docetaxel), podophylotoxins or vinca alkaloids (vincristine, vinblastine);

b) antimetabolite drugs such as 5-fluorouracil, cytarabine, gemcitabine, purine analogues such as pentostatin, methotrexate);

c) alkylating agents such as nitrogen mustards (such as cyclophosphamide or ifosphamide);

d) drugs which target DNA such as the antracycline drugs adriamycin, doxorubicin, pharmorubicin or epirubicin;

e) drugs which target topoisomerases such as etoposide;

f) hormones and hormone agonists or antagonists such as estrogens, antiestrogens (tamoxifen and related compounds) and androgens, flutamide, leuprorelin, goserelin, cyprotrone or octreotide;

g) drugs which target signal transduction in tumour cells including antibody derivatives such as herceptin;

h) alkylating drugs such as platinum drugs (cis-platin, carbonplatin, oxaliplatin, paraplatin) or nitrosoureas;

i) drugs potentially affecting metastasis of tumours such as matrix metalloproteinase inhibitors;

j) gene therapy and antisense agents;

k) antibody therapeutics;

l) other bioactive compounds of marine origin, notably the ecteinascidins such as ecteinascidin 743 or the didemnins such as aplidine;

m) steroid analogues, in particular dexamethasone;

n) anti-inflammatory drugs, in particular dexamethasone; and

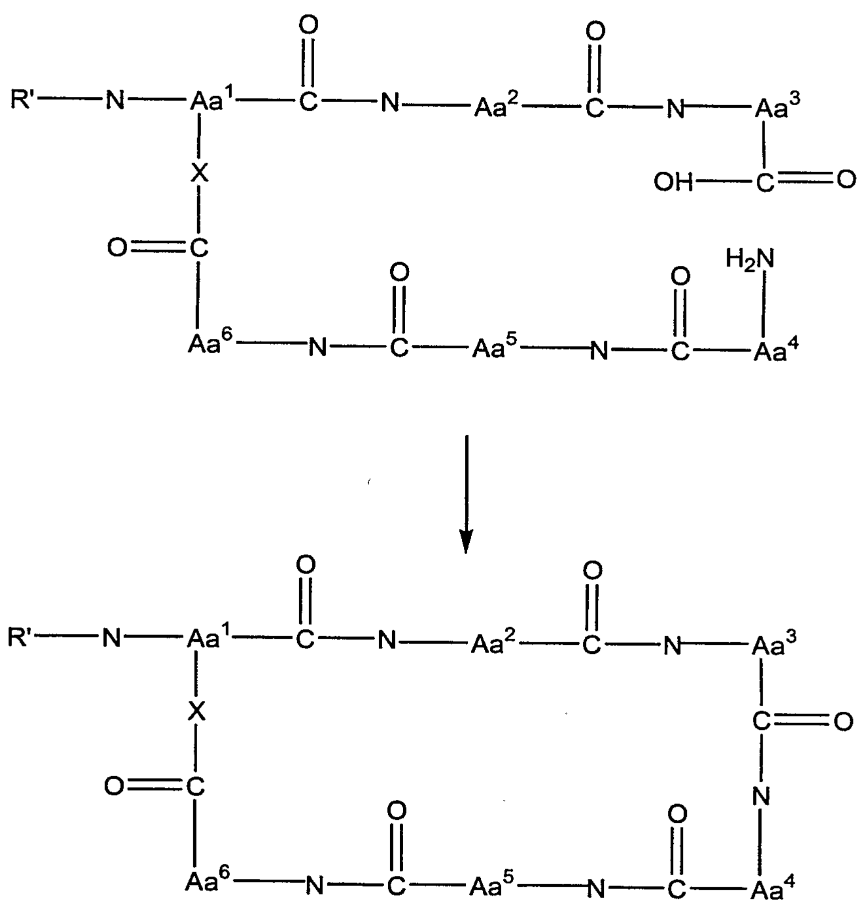
o) anti-emetic drugs, in particular dexamethasone.

The present invention also extends to the compounds of the invention for use in a method of treatment, and to the use of the compounds in the preparation of a composition for treatment of cancer.

Compounds of this invention are also expected to have antituberculosis activity.

According to this invention there is also provided a process for preparing compounds of this invention including kahalalide F, which comprises ring closing between Aa_3 and Aa_4. We have found that ring closing at this position gives a superior process. Ring closing can be preceded and/or followed by other modifications to the molecule, including forming or extending the side chain on Aa_1, and/or removing protecting groups and/or modifying substituents on the amino acids.

Thus, the process can be illustrated by the following scheme:

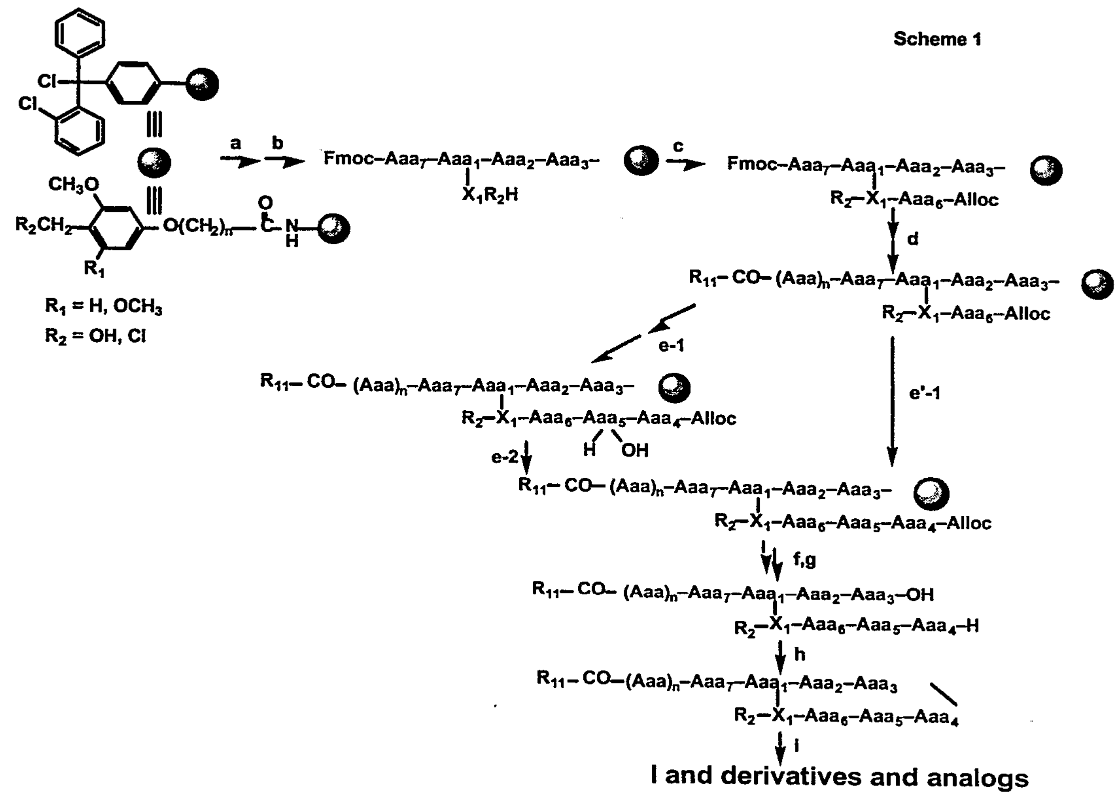


where R' is a group R-CO-N- or a precursor therefor, and where one or more of the amino acids may have protecting groups, and where the -COOH of Aa^3

and/or the $-NH_2$ of Aa^4 may optionally be protected, activated or derivatised. Such procedures using precursors or of protection, activation or derivatisation are in themselves entirely conventional, and further details are not needed to practice the process.

The process of this invention can be carried out from starting materials in an enantio- and stereocontrolled and fast manner, taking advantages of the solid-phase synthetic methodology, where the molecule in construction is bounded to an insoluble support during all synthetic operations. Thus, excess of reagents and soluble by-products can be removed simply by washing the molecule-resin with suitable solvents. Large excesses of the soluble reagents can, therefore, be used in order to drive the reactions to completion in a short period of time, avoiding racemization (if applies) and other secondary reactions. The method is also amenable for automation.

The preferred embodiment of the synthetic process of the present invention is best represented in the Scheme 1, which is directed to the formation of compounds of the formula (II).



Scheme 1

As shown above in Scheme 1, the preferred process for the synthetic formation of Kahalalide F (I) and derivatives and analogues is based in a solid-phase approach, see for example Lloyd-Williams, P., *et al.*, *Chemical Approaches to the Synthesis of Peptides and Proteins*, CRC Press, Boca Raton (FL), 1997.

The process of Scheme 1 comprises the sequential steps of:

(a) incorporating an Fmoc-amino acid (Aaa3) onto a solid support (*e.g.*, polystyrene, polyethylene grafted on polystyrene, and the like) containing a super-acid labile handle or linker (*e.g.*, chlorotrityl, polyalkoxybenzyl, and the like) forming an ester bond;

(b) elongating the peptidic chain with three amino acids (Aaa_2, Aaa_1, Aaa_7) using a Fmoc/*t*Bu strategy. For I, X_1 = OH and introduced without protection. When $X_1 = NH_2$, this is introduced with Alloc protection;

(c) incorporating (Aaa_6) using an Alloc/*t*Bu strategy;

(d) elongating the peptidic chain with the remaining amino acids and R_{11}-COOH through Aaa_7 using a Fmoc/*t*Bu strategy;

(e-1) elongating the peptidic chain with two amino acids [Aaa_5 (OH,H), Aaa_4] through Aaa_6 using an Alloc/*t*Bu strategy. The OH attached to Aaa_5 is unprotected;

(e-2) dehydrating in solid-phase to give the peptide with Aaa_5;

or

(e'-1) incorporating the dipeptide Alloc-Aaa_4–Aaa_5–OH, which has been combined and dehydrated in solution;

(f) removing the Alloc group of Aaa_4 while the peptide is still anchored to the solid support;

(g) cleaving the side-chain protected, if applies, peptide from the solid support;

(h) cyclizing the peptide in solution;

(i) removing TFA labile side chain protecting groups.

It will be appreciated that the particular choice of protecting groups is not critical, and other choices are widely available. For example, Bzl type groups can replace tBu/Boc; Boc instead of Fmoc; Fmoc instead of Alloc; Wang resin instead of chlorotrityl.

DETAILED DESCRIPTION OF THE PREFERRED EMBODIMENTS OF THE PROCESS

The preferred process of the present invention is illustrated in Scheme 1. As shown therein, and as discussed in greater detail in the examples which follow below, this process was conducted as follows:

Fmoc-Aaa3-OH was incorporated preferably to a chlorotrityl-polystyrene resin, see Barlos, K.; Gatos, D.; Schäfer, W. Angew. Chem. Int. Ed. Engl. 1991, 30, 590-593, in the presence of DIEA keeping the level of substitution of aprox. 0.15-0.5 mmol/g. The use of higher loadings brings the presence of terminated peptides in the final product, see Chiva, C.; Vilaseca, M.; Giralt, E.; Albericio, F. J. Pept. Sci. 1999, 5, 131-140.

Removal of the Fmoc group was carried out with piperidine–DMF (2:8, v/v) (1 x 1 min, 3 x 5 min, 1 x 10 min). Couplings of Fmoc-, Alloc-Aaa-OH, and R11-COOH (5 equiv) were carried out with HATU–DIEA, see Carpino, L.A.; El-Faham, A.; Minor, C.A.; Albericio, F. J. Chem. Soc., Chem. Commun. 1994, 201-203, (5:10) in DMF for 90 min. After the coupling ninhydrin test was carried out and if it was positive the coupling was repeated in the same conditions, otherwise the process was continued. Washings between deprotection, coupling, and, again, deprotection steps were carried out with DMF (5 x 0.5 min) and CH_2Cl_2 (5 x 0.5 min) using each time 10 mL solvent/g resin.

Incorporation of Alloc-Aaa6-OH (7 equiv) when X1 = O was carried with equimolar amount of DIPCDI and 0.7 equiv of DMAP for 2 h. This coupling was repeated twice.

Removal of Alloc group was carried out with Pd(PPh3)4 (0.1 equiv) in the presence of PhSiH3 (10 equiv) under atmosphere of Ar, see Gómez-Martínez, P.; Thieriet, N.; Albericio, F.; Guibé, F. J. Chem. Soc. Perkin I 1999, 2871-2874.

Dehydration was carried out in solid-phase with EDC (water soluble carbodiimide, 100 equiv) in the presence of CuCl (60 equiv) in CH2Cl2–DMF (10:2) for 6 days. EDC/CuCl has been used by dehydration in solution of a residue of Thr in a fragment of Nisin. Fukase, K.; Kitazawa, M.; Sano, A.; Shimbo, K.; Horimoto, S.; Fujita, H.; Kubo, A.; Wakamiya, T.; Shibe, A. Bull. Chem. Soc. Jpn. 1992, 65, 2227-2240.

The dipeptide Alloc-Aaa4–Aaa5–OH (5 equiv), which was prepared in solution from Alloc-Aaa4-OH and H-Aaa5(OH, H)-O*t*Bu with EDC, and posterior dehydratation and treatment with TFA, was coupled with HATU–DIEA (5:10) for 16 h and recoupling for 3 h The use of other coupling reagents based in HOBt, such as HBTU or DIPCDI–HOBt, have led to incomplete incorporations of the dipeptide.

Cleavage of the protected peptide from the resin was accomplished by TFA–CH_2Cl_2 (1:99) (5 x 30 sec).

Cyclization step was carried out with DIPCDI–HOBt (3:3 equiv) in CH2Cl2–DMF for 2 hrs. Other methods, such as PyBOP/DIEA (3:6 equiv) in DMF can be also be used.

Final deprotection was carried out with TFA–H_2O (95:5) for 1 h.

EXAMPLES OF THE INVENTION

Abbreviations

Abbreviations used for amino acids and the designations of peptides follow the rules of the IUPAC-IUB Commission of Biochemical Nomenclature in *J. Biol. Chem.* 1972, *247*, 977-983. The following additional abbreviations are used: 4-AcBut-OH, 4-acetoxybutyric acid; AcButBut-OH, 4-(4-acetoxybutanoyloxy)-butyric acid; ACH, a-cyano-4-hydroxycinnamic acid; Alloc, allyloxycarbonyl; Boc, *tert*-butyloxycarbonyl; *t*-Bu, *tert*-Butyl; But-OH, butyric acid; Cl-TrtCl-resin, 2-chlorotrityl chloride-resin; Dap, 2,3-diaminopropionic acid; 4-DiMeABut-OH, *N,N*-dimethyl-4-aminobutyric acid; 3,3-DiMeBut-OH, 3,3-Dimethylbutyric acid; DHB, 2,5-dihydroxybenzoic acid; *Z*-Dhb, *a,b*-Didehydro-a-aminobutyric acid; DIEA, *N,N*-diisopropylethylamine; DMF, *N,N*-dimethylformanide; EDC, 1-ethyl-3-(3'-dimethylaminopropyl)carbodiimide hydrochloride; Etg, ethylglycine; ESMS, electrospray mass spectrometry; FABMS, fast atom bombardment mass spectrometry; Fmoc, 9-fluorenylmethoxycarbonyl; HATU, *N*-[(dimethylamino)-1*H*-1,2,3-triazolo-[4,5-*b*]pyridin-1-yl-methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; HBTU, *N*-[(1*H*-benzotriazol-1-yl)(dimethylamino)methylene]-*N*-methylmethanaminium hexafluorophosphate *N*-oxide; 2-Hedo-OH, 2,4-hexadienoic acid; Hep-OH, heptanoic acid; HOAc, acetic acid, HOAt, 1-hydroxy-7-azabenzotriazole (3-hydroxy-3*H*-1,2,3-triazolo-[4,5-*b*]pyridine); HOBt, 1-hydroxybenzotriazole; IBut-OH, isobutyric acid; 3-MeBut-OH, 3-methylbutyric acid; 5-MeHex-OH, 5-methylhexanoic acid; MeOH, methanol; 4-MePen-OH, 4-methylpentanoic acid; NMM, *N*-methylmorpholine; 4-HOBut, 4-hydroxybutyric; (b-OH)Phe-OH, b-hydroxyphenylalanine; Lit-OH, Lithocholic acid; Pal, palmitic acid; D-Phe-OH, *a,b*-Didehydrophenylalanine; PyAOP, 7-azabenzotriazol-1-yl-*N*-oxy-

tris(pyrrolidino)phosphonium hexafluorophosphate; PyBOP, benzotriazol-1-yl-*N*-oxy-tris(pyrrolidino)phosphonium hexafluorophosphate; SPS, solid-phase synthesis; TFA, trifluoroacetic acid; Tico-OH, tetraicosanoic acid. Amino acid symbols denote the L-configuration unless stated otherwise. All solvent ratios are volume/volume unless stated otherwise.

General Procedures. Cl-TrtCl-resin, Protected Fmoc-amino acid derivatives, HOBt, PyBOP, HATU were from PerSeptive Biosystems (Framingham, MA), Bachem (Bubendorf, Switzerland), NovaBiochem (Läufelfingen, Switzerland). Alloc-amino acids were preparated essentially as described by Dangles *et al.*, see Dangles, O.; Guibé, F.; Balavoine, G.; Lavielle, S.; Marquet. A. J. Org. Chem. 1987, 52, 4984-4993, and 5-MeHex-OH through a malonic synthesis. DIEA, DIPCDI, EDC, Piperidine, TFA were from Aldrich (Milwaukee, WI). DMF and CH_2Cl_2 were from SDS (Peypin, France). Acetonitrile (HPLC grade) was from Scharlau (Barcelona, Spain). All comercial reagents and solvents were used as received with exception of DMF and CH_2Cl_2, which were bubbled with nitrogen to remove volatile contaminants (DMF) and stored over activated 4 Å molecular sieves (Merck, Darmstadt, Germany) (DMF) or $CaCl_2$ (CH_2Cl_2). Solution reactions were performed in round bottom flasks. Organic solvent extracts were dried over anhydrous $MgSO_4$, followed by solvent removal at reduced pressures and <40 °C.

Solid-phase synthesis were carried out in polypropylene syringes (20, 10 mL) fitted with a polyethylene porous disc. Solvents and soluble reagents were removed by suction. Removal of the Fmoc group was carried out with piperidine–DMF (2:8, v/v) (1 x 1 min, 3 x 5 min, 1 x 10 min). Washings between deprotection, coupling, and, again, deprotection steps were carried out with DMF (5 x 0.5 min) and CH_2Cl_2 (5 x 0.5 min) using

each time 10 mL solvent/g resin. Peptide synthesis transformations and washes were performed at 25 °C. Syntheses carried out on solid-phase were controlled by HPLC of the intermediates obtained after cleaving with TFA–H_2O (9:1) for 60 min an aliquot (aprox. 10 mg) of the peptidyl-resin HPLC columns [Kromasil C_{18} (Conditions A-F) /C_4 (Conditions G,H)] reversed-phase column, 4.6 x 250 mm, 10 mm) were from Akzo Nobel (Bohum, Sweden). Analytical HPLC was carried out on a Shimadzu instrument comprising two solvent delivery pumps (model LC-6A), automatic injector (model SIL-6B), variable wavelength detector (model SPD-6A), system controller (model SCL-6B) and plotter (model C-R6A). UV detection was at 220 nm, and linear gradients of CH_3CN (+0.036% TFA) into H_2O (+0.045% TFA) were run at 1.0 mL/min flow rate from: (Condition A) 1:9 to 10:0 over 30 min; (Condition B) 3:7 to 10:0 over 30 min; (Condition C) 1:19 to 19:1 over 30 min; (Condition D) 45:55 to 90:10 over 30 min; (Condition E) 45:55 to 6:4 over 30 min; (Condition F) isocratic 1:1; (Condition G) 3:7 to 1:0 over 30 min; (Condition H) 1:1 to 1:0 over 30 min.

MALDI-TOF- and ES-MS analysis of peptide samples were performed in a PerSeptive Biosystems Voyager DE RP, using CHCA or DHB matrices, and in a Micromass VG-quattro spectrometer. Peptide-resin samples were hydrolyzed in 12 N aqueous HCl-propionic acid (1:1), at 155 °C for 1—3 h and peptide-free samples were hydrolyzed in 6 N aqueous HCl at 155 °C for 1 h. Subsequent amino acid analyses were performed on a Beckman System 6300 autoanalyzer. ^{1}H-NMR (500 MHz, 200 MHz) and ^{13}C-NMR (50 MHz) spectroscopy was performed on a Bruker DMX-500 (11.7 T) and Varian Gemini 200 (4.7 T). Chemical shifts (d) are expressed in parts per million downfield from TMS. Coupling constants are expressed in hertz.

Kahalalide F (I)

Example 1

H-D-Val-O-TrtCl-resin.

Cl-TrtCl-resin (1 g, 1.35 mmol/g) was placed in a 20 mL polypropylene syringe fitted with a polyethylene filter disk. The resin was then washed with CH_2Cl_2 (5 x 0.5 min), and a solution of Fmoc-D-Val-OH (92 mg, 0.27 mmol, 0.2 equiv) and DIEA (471 mL, 2.7 mmol, 2 equiv) in CH_2Cl_2 (2.5 mL) was added, and the mixture was stirred for 1 h. The reaction was terminated by addition of MeOH (800 mL), after a stirring of 15 min. The Fmoc-D-Val-O-TrtCl-resin was subjected to the following washings/treatments with CH_2Cl_2 (3 x 0.5 min), DMF (3 x 0.5 min), piperidine-CH_2Cl_2-DMF (1:9.5:9.5, 1 x 10 min), piperidine-DMF (1:4, 1 x 15 min), DMF (5 x 0.5 min), isopropanol (2 x 1 min), DMF (5 x 0.5 min), MeOH (2 x 1 min), and dried over vacuum. The loading as calculated by AAA was 0.15 mmol/g.

Example 2

Fmoc-D-*allo*-Ile-D-*allo*-Thr(Val-Alloc)-D-*allo*-Ile-D-Val-O-TrtCl-resin.

Fmoc-D-*allo*-Ile-OH (265 mg, 0.75 mmol, 5 equiv), Fmoc-D-*allo*-Thr-OH (free hydroxy group) (256 mg, 0.75 mmol, 5 equiv), and Fmoc-D-*allo*-Ile-OH (265 mg, 0.75 mmol, 5 equiv) were added sequentially to the above obtained H-D-Val-O-TrtCl-resin using HATU (285 mg, 0.75 mmol, 5 equiv) and DIEA (261mL, 1.5 mmol, 10 equiv) in DMF (2.5 mL). In all cases, after 90 min of coupling, the ninhydrin test was negative. Removal of Fmoc group and washings were carried out as described in General Procedures. Alloc-Val-OH (211 mg, 1.05 mmol, 7 equiv) was coupled with DIPCDI (163 mg, 1.05 mmol, 7 equiv) in the presence of DMAP (12.8 mg, 0.105 mmol, 0.7 equiv). This coupling was repeated in the same conditions twice. An

aliqout of the peptidyl-resin was treated with TFA–H_2O (9:1) for 60 min and the HPLC (Condition A, t_R 25.9 min) of the crude obtained after evaporation showed a purity of > 98%. ESMS, calcd for $C_{45}H_{63}N_5O_{11}$, 849.5. Found: *m/z* 850.1 $[M+H]^+$.

Example 3

5-MeHex-D-Val-Thr(*t*Bu)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-Alloc)-D-*allo*-Ile-D-Val-O-TrtCl-resin.

The Fmoc group was removed and Fmoc-Orn(Boc)-OH (341 mg, 0.75 mmol, 5 equiv), Fmoc-D-Pro-OH (253 mg, 0.75 mmol, 5 equiv), Fmoc-D-Val-OH (255 mg, 0.75 mmol, 5 equiv), Fmoc-Val-OH (255 mg, 0.75 mmol, 5 equiv), Fmoc-Thr(*t*Bu)-OH (298 mg, 0.75 mmol, 5 equiv), Fmoc-D-Val-OH (255 mg, 0.75 mmol, 5 equiv), and 5-MeHex-OH (98 mg, 0.75 mmol, 5 equiv) were sequentially added to the above peptidyl-resin (Example 2) using HATU (285 mg, 0.75 mmol, 5 equiv) and DIEA (261 mL, 1.5 mmol, 10 equiv) in DMF (2.5 mL). In all cases, after 90 min of coupling, the ninhydrin or chloranil (after incorporation of D-Val) tests were negative. Removal of Fmoc group and washings were carried out as described in General Procedures. An aliqout of the peptidyl-resin was treated with TFA–H_2O (9:1) for 60 min and the HPLC (Condition B, t_R 17.2 min) of the crude obtained after evaporation showed a purity of > 82%. ESMS, calcd for $C_{66}H_{116}N_{12}O_{17}$, 1,348.9. Found: *m/z* 1,350.0 $[M+H]^+$.

Example 4

5-MeHex-D-Val-Thr(*tBu*)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-Thr-Phe-Alloc)-D-*allo*-Ile-D-Val-O-TrtCl-resin.

Alloc group was removed with $Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol, 0.1 equiv) in the presence of $PhSiH_3$ (185 µL, 1.5 mmol, 10 equiv) under atmosphere of Ar. Fmoc-Thr-OH (free hydroxy group) (256 mg, 0,75 mmol, 5 equiv) and Alloc-Phe-OH (187 mg, 0,75 mmol, 5 equiv) were added sequentially to the above peptidyl-resin (Example 3) using HATU (285 mg, 0.75 mmol, 5 equiv) and DIEA (261 mL, 1.5 mmol, 10 equiv) in DMF (2.5 mL). In all cases, after 90 min of coupling, the ninhydrin test was negative. Removal of Fmoc group and washings were carried out as described in General Procedures. An aliqout of the peptidyl-resin was treated with TFA–H_2O (9:1) for 60 min and the HPLC of the crude obtained after evaporation showed a purity by HPLC (Condition B, t_R 17.8 min) of > 70%. ESMS, calcd for C_{79} H_{132} N_{14} O_{20}, 1,597.0. Found: *m/z* 1,598.2 $[M+H]^+$.

Example 5

5-MeHex-D-Val-Thr(*tBu*)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-*Z*-Dhb-Phe-H)-D-*allo*-Ile-D-Val-O-TrtCl-resin (*via* dehydration in solid-phase).

The above peptidyl-resin (Example 4) was treated with EDC (2.88 g, 15 mmol, 100 equiv), CuCl (891 mg, 9 mmol, 60 equiv) in CH_2Cl_2–DMF (10:2) (12 mL) for 6 days. After extensively washings with DMF, CH_2Cl_2, and DMF, the Alloc group was removed with $Pd(PPh_3)_4$ (17.3 mg, 0.015 mmol, 0.1 equiv) in the presence of $PhSiH_3$ (185 µL, 1.5 mmol, 10 equiv) under atmosphere of Ar. The final loading as calculated by AAA was 0.11 mmol/g (92% overall yield). An aliquot of the peptidyl-resin was treated with TFA–H_2O (9:1) for 60 min and the HPLC (Condition B, t_R 17.2 min) of the crude obtained after evaporation showed a purity by of > 70%. ESMS, calcd for C_{75} H_{126} N_{14} O_{17}, 1495.0 Found: *m/z* 1,496.0 $[M+H]^+$.

Example 6

5-MeHex-D-Val-Thr(*tBu*)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-*Z*-Dhb-Phe-H)-D-*allo*-Ile-D-Val-OH.

The protected peptide was cleaved from the resin (0. 5 g, 55.9 μmol) by TFA–CH_2Cl_2 (1:99) (5 x 30 sec). The combined filtrates were evaporated to dryness under reduced pressure and lyophilized, to give 80.2 mg (48.5 μmol, 87% yield) of the title compound with a purity of > 70% as checked by HPLC (Condition B, t_R 25.7 min). ESMS, calcd for C_{84} H_{142} N_{14} O_{19}, 1,651.1. Found: *m/z* 1,652.3 $[M+H]^+$.

Example 7

Kahalalide F (I).

The protected peptide (Example 6) (40.0 mg, 24 μmol) was dissolved in DMF (25 mL), and PyBOP (37.8 mg, 73 μmol, 3 equiv) and DIEA (25 mL, 145 μmol, 6 equiv) were added. The mixture was allowed to stir for 1 h, and then the solvent was removed by evaporation under reduced pressure. The protected cyclic peptide was dissolved in TFA–H_2O (19:1, 5 mL) and the mixture was allowed to stir for 1 h. The solvent was removed by evaporation under reduced pressure, and then H_2O (5 mL) was added and lyophilized. The crude product was purified by medium pressure chromatography (Vydac C_{18} 15-20 μm, 300 Å, 240 x 24 mm), linear gradient from 20% to 60% of acetonitrile (+0.05% TFA) in water (+0.05% TFA) in 5 h (300 mL each solvent), 120 mL/h, detection at 220 nm, to give the title product (5.0 mg, 3.4 μmol, 14% yield). MALDI-TOF-MS, calcd for C_{75} H_{124} N_{14} O_{16}, 1,477.9. Found: *m/z* 1,478.7 $[M+H]^+$, 1,500.6 $[M+Na]^+$, 1,516.5 $[M+K]^+$. The product co-eluted by HPLC [Condition D (t_R 12.5 min), E (t_R 17.4 min),

and F(t_R 12.1 min)] with an authentic sample of Kahalalide F. The ^{1}H-NMR (500 MHz, d^6-DMSO) spectrum of the compound was identical to the natural product (data shown in Table I).

Table I

RESIDUE	N-H	Hα	Hβ	OTHER
(*Z*)-Dhb	9.69 (s)	-	6.34 (q, J =7.0 Hz)	1.26 (d, J =7.5 Hz, γ-CH_3)
D-al·lo-Ile 1	8.82 (d, J =10.0 Hz)	4.31	1.73	1.31, 1.02, 0.77 (γ-CH_2, γ-CH_3, δ-CH_3)
L-Phe	8.79 (d, J =5.5 Hz)	4.42	2.93 (m)	7.20 (1 H Ar, m) 7.28 (4H Ar, m)
D-al·lo-Thr	8.56 (d, J =8.0 Hz)	4.53	4.96 (m)	1.07 (δ, J = 6.5 Hz, γ-CH_3)
D-Val 3	8.10 (d, J =8.5 Hz)	4.26	1.94	0.86 (2 γ-CH_3)
L-Orn	7.95 (d, J =8.5 Hz)	4.49	1.48 (2H)	1.67 (γ-CH_2), 2.74 (bs, δ-CH_2), 7.69 (ε-NH_3^+)
D-al·lo-Ile 2	7.90 (d)	4.37	1.69	1.30, 1.03, 0.77 (γ-CH_2, γ-CH_3, δ-CH_3)
D-Val 5	7.88 (d)	4.23	1.96	0.84 (2 γ-CH_3)
L-Thr	7.82 (d, J =8.0 Hz)	4.26	3.97 (m)	4.88 (d, J =5.0 Hz, OH), 0.98 (d, J =6.5 Hz, γ-CH_3)

D-Val 2	7.62 (d, J =8.5 Hz)	4.46	2.17	0.77 (γ-CH_3), 0.62 (d, J =7.0 Hz, γ-CH_3)
L-Val 4	7.57 (d, J =8.5 Hz)	4.28	1.98	0.80 (2 γ-CH_3)
L-Val 1	6.76 (d, J =9.0 Hz)	3.86	1.39	0.62 (d, J =7.0 Hz, γ-CH_3), 0.58 (d, J =6.0 Hz, γ-CH_3)
D-Pro	-	4.36	2.03, 1.87, 1.79 (β-CH_2, γ-CH_2), 3.76 (1H, m, δ-CH_2), 3.53 (1H, m, -CH_2)	
5-MeHex	-	2.13 (2H)	1.47 (β-CH_2, δ-CH), 1.11 (γ-CH_2), 0.82 (2 ε-CH_3)	

Example 7bis

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 7. The product was characterized by HPLC (t_R 17.4 min, Condition B) and ESMS, calcd for C_{75} H_{124} N_{14} O_{16}, 1,477.9. Found: *m/z* 1,501.3 $[M+Na]^+$, 1,517.3 $[M+K]^+$.

Kahalalide F (I)

Example 8

Alloc-Phe-Thr-O*t*Bu.

H-Thr-O*t*Bu·HCl (3.1 g, 15 mmol, 1.3 equiv) was dissolved in CH_2Cl_2 (30 mL) and DIEA (2.9 mL, 17 mmol, 1.5 equiv) was added and the mixture allowed to stir for 30 min. Alloc-Phe-OH (2.8 g, 11 mmol, 1 equiv) and EDC (2.8 g, 15 mmol, 1.3 equiv) in CH_2Cl_2 (35 mL) was then added and the reaction was stirred for 18 h. The organic reaction mixture was washed with H_2O (3 x 25 mL), dried ($MgSO_4$) and concentrated *in vacuo*. The resultant oil (4.12 g) was purified by flash chromatography [$CHCl_3$–MeOH–HOAc (9:1:0.2)] to give the title product (3.25 g, 8 mmol, 71% yield), which was characterized by analytical HPLC (t_R 20.9 min, > 98% purity; Condition C); ESMS, calcd for $C_{21}H_{30}N_2O_6$, 406.2. Found: *m/z* 408.0 $[M+H]^+$; ^{1}H-NMR (200 MHz, $CDCl_3$): 7.1-7.3 (5 H, m, Ar); 6.89 (1 H, d, J = 8.8 Hz, NH); 5.7-5.9 (1 H, m, CH allyl); 5.56 (1 H, d, J = 8.0 Hz, OH); 5.2-5.3 (2 H, m, CH_2 g-allyl); 4.52 (2 H, d, J = 5.4 Hz, CH_2 a-allyl); 4.45 (1 H, dd, J = 8.4 Hz, 2.8 Hz, a-CH Thr); 4.22 (1 H, dq, J = 6.2 Hz, J = 3.0 Hz, b-CH Thr); 3.0-3.2 (2 H, m, b-CH_2 Phe); 1.47 (9 H, s, *t*Bu); 1.15 (3 H, d, J = 6.6 Hz, g-CH_3 Thr); ^{13}C-NMR (50 MHz, $CDCl_3$): 172.2 (CO), 169.5 (CO); 156.0 (CO); 136.1 (Cq, Ar); 132.5 (CH, allyl); 129.3 (CH, Ar); 128.5 (CH, Ar); 126.9 (CH, Ar); 117.7 (CH_2, allyl); 82.7 (Cq, *t*Bu); 68.5 (b-CH Thr); 65.9 (CH_2, allyl); 58.0 (a-CH Thr); 56.2 (a-CH Phe); 38.3 (b-CH_2 Phe); 28.0 (CH_3, *t*Bu); 20.8 (g-CH_3 Thr).

Example 9

Alloc-Phe-*Z*-Dhb-O*t*Bu.

Alloc-Phe-Thr-O*t*Bu (3.25 g, 8.0 mmol), EDC (9.90 g, 52 mmol, 6.5 equiv), and CuCl (2.14 g, 22 mmol, 2.7 equiv) were dissolved in CH_2Cl_2–

anhydrous DMF (65 ml, 12:1) under N_2 and the mixture was allowed to stir for 2 days under N_2. The organic solvent was removed *in vacuo* and the residue was taken in a saturated solution of EDTA (100 mL), which was extracted with EtOAc (3 x 50 mL). The combined organic solution was washed with brine (3 x 60 mL) dried ($MgSO_4$) and concentrated *in vacuo* to give a pale yellow solid (2.8 g), was purified by flash chromatography [$CHCl_3$–MeOH–HOAc (9:1:0.1)] to give the title product (2.6 g, 6.7 mmol, 84% yield), which was characterized by analytical HPLC (t_R 23.1 min, > 95% purity; Condition C); ESMS, calcd for $C_{21}H_{28}N_2O_5$, 388.2. Found: m/z 389.6 $[M+H]^+$; ^{1}H-NMR (200 MHz, $CDCl_3$): 7.2-7.4 (5 H, m, Ar); 6.69 (1 H, q, J = 7.4 Hz, CH Dhb); 5.8-6.0 (1 H, m, CH allyl); 5.2-5.3 (2 H, m, CH_2 g-allyl); 4.5-4.6 (2 H, m, a-CH_2 allyl); 3.1-3.3 (2 H, m, b-CH_2 Phe); 1.67 (3 H, d, J = 7.2 Hz, CH_3 Dhb); 1.47 (9 H, s, *t*Bu); ^{13}C-NMR (50 MHz, $CDCl_3$): 168.9 (CO); 163.1 (CO); 156.0 (CO); 136.0 (Cq, Ar); 132.6, 132.3 (CH, allyl; b-CH Dhb); 129.3 (CH, Ar); 128.7 (CH, Ar); 127.0 (CH, Ar); 117.9 (CH_2, allyl); 81.7 (Cq, *t*Bu); 66.0 (CH_2, allyl); 56.3 (a-CH Phe); 38.2 (b-CH_2 Phe); 28.0 (CH_3, *t*Bu); 14.7 (CH_3 Dhb).

Example 10

Alloc-Phe-*Z*-Dhb-OH.

Alloc-Phe-*Z*-Dhb-O*t*Bu (2.6 g, 6.7 mmol) was dissolved in TFA–CH_2Cl_2–H_2O (90:8:2, 5.5 mL) and the mixture was allowed to stir for 3 h. The organic reaction was concentrated *in vacuo* to give the title product (2.2 g, 6.6 mmol, 99% yield), which was characterized by analytical HPLC (t_R 17.0 min, > 95% purity; Condition C); ESMS, calcd for $C_{17}H_{20}N_2O_5$, 332.1. Found: m/z 333.7 $[M+H]^+$; ^{1}H-NMR (200 MHz, CD_3OD): 7.2-7.3 (5 H, m, Ar); 6.84 (1 H, q, J = 7.2 Hz, CH Dhb); 5.8-6.0 (1 H, m, CH allyl); 5.1-

5.3 (2 H, m, CH_2 g-allyl); 4.4-4.5 (2 H, m, a-CH_2 allyl); 3.2-3.3 (1 H, m, b-CH_2 Phe); 2.8-3.0 (1 H, m, b-CH_2 Phe); 1.66 (3 H, d, J = 7.4 Hz, CH_3 Dhb); ^{13}C-NMR (50 MHz, CD_3OD): 173.1 (CO); 138.5 (Cq, Ar); 136.7, 134.1 (CH, allyl; b-CH Dhb); 130.3 (CH, Ar); 129.4 (CH, Ar); 127.7 (CH, Ar); 117.4 (CH_2, allyl); 66.6 (CH_2, allyl); 57.8 (a-CH Phe); 39.1 (b-CH_2 Phe); 14.1 (CH_3 Dhb).

Example 11

5-MeHex-D-Val-Thr(*tBu*)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-Alloc)-D-*allo*-Ile-D-Val-O-TrtCl-resin.

Cl-TrtCl-resin (0.45 g, 1.35 mmol/g) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disk. The peptidyl-resin was obtained following the same procedure described in Examples 1-3 except that in the coupling of the first aminoacid (Fmoc-D-Val-OH) to the resin 0.3 equiv instead of 0.2 equiv were used. The initial loading as calculated by AAA was 0.29 mmol/g. Once the aminoacids were coupled, an aliquot of the peptidyl-resin was treated with TFA–H_2O (9:1) for 60 min and the HPLC (Condition B, t_R 17.3 min) of the crude obtained after evaporation showed a purity of > 80%. ESMS, calcd for $C_{66}H_{116}N_{12}O_{17}$, 1,348.9. Found: *m/z* 1,350.1 $[M+H]^+$.

Example 12

5-MeHex-D-Val-Thr(*tBu*)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-*Z*-Dhb-Phe-Alloc)-D-*allo*-Ile-D-Val-O-TrtCl-resin (*via* incorporation of the dipeptide).

The peptidyl-resin was treated with $Pd(PPh_3)_4$ (15.1 mg, 0.013 mmol, 0.1 equiv) in the presence of $PhSiH_3$ (161 μL, 1.3 mmol, 10 equiv) under

atmosphere of Ar, for removing the Alloc group. Alloc-Phe-*Z*-Dhb-OH (217 mg, 0.65 mmol, 5 equiv) and HATU (249 mg, 0.65 mmol, 5 equiv) were dissolved in DMF (1.25 mL) and added to peptidyl-resin, then DIEA (228 μL, 1.3 mmol, 10 equiv) was added and the mixture stirred overnight. After washings, the coupling was repeated with the same amount of reagents for 3 h., when the ninhydrin test was negative. After washings with DMF and CH_2Cl_2, an aliquot of the peptidyl-resin was treated with TFA–H_2O (9:1) for 60 min and the HPLC (Condition B, t_R 18.3 min) of the crude obtained after evaporation showed a purity by of > 80%. MALDI-TOF-MS, calcd for C_{79} H_{130} N_{14} O_{19}, 1,579.0. Found: *m/z* 1,580.3 $[M+H]^+$, 1,602.2 $[M+Na]^+$, 1,618.2 $[M+K]^+$.

Example 13

5-MeHex-D-Val-Thr(*tBu*)-Val-D-Val-D-Pro-Orn(Boc)-D-*allo*-Ile-D-*allo*-Thr(Val-*Z*-Dhb-Phe-H)-D-*allo*-Ile-D-Val-OH.

The Alloc group from peptidyl-resin (Example 12) was removed with $Pd(PPh_3)_4$ (15.1 mg, 0.013 mmol, 0.1 equiv) in the presence of $PhSiH_3$ (161 μL, 1.3 mmol, 10 equiv) under atmosphere of Ar. The final loading as calculated by AAA was 0.16 mmol/g (79% overall yield). The protected peptide was cleaved from the resin (235 mg, 37.5 mmol) by TFA–CH_2Cl_2 (1:99) (5 x 30 sec). The combined filtrates were evaporated to dryness under reduced pressure and lyophilized, to give 40.5 mg (24.5 mmol, 65%) of the title compound with a purity > 80% as checked by HPLC (Condition B, t_R 24.3 min). MALDI-TOF-MS, calcd for C_{84} H_{142} N_{14} O_{19}, 1,651.1. Found: *m/z* 1,674.8 $[M+Na]^+$, 1,690.8 $[M+K]^+$.

Example 14

Kahalalide F (I).

The protected peptide (Example 13) (38.5 mg, 23 μmol) was dissolved in DMF (25 mL), and PyBOP (36.4 mg, 70 μmol, 3 equiv) and DIEA (24 mL, 140 μmol, 6 equiv) were added. The mixture was allowed to stirr for 1 h, and then the solvent was removed by evaporation under reduced pressure. The protected cyclic peptide was dissolved in TFA–H_2O (19:1, 5 mL) and the mixture was allowed to stir for 1 h. The solvent was removed by evaporation under reduced pressure, and then H_2O (5 mL) was added and lyophilized. The crude product was purified as described in Example 7 to give the title product (3.6 mg, 2.4 μmol, 10% yield), which was identical to the one obtained in Example 7.

5-MeHex-D-Val-Thr-D-Val-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val) (structure assigned to Kahalalide F by Goetz, G., et al. *Tetrahedron,* 1999, vol. 55, pp. 7739-7746)

Example 15

5-MeHex-D-Val-Thr-D-Val-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val) (*via* dehydration in solid-phase).

Experimental procedures as decribed in Examples 1-7 were carried out with the only exception that, in Example 3, Fmoc-Val-OH was incorporated to D-Pro-peptidyl-resin and then after removal of the Fmoc group, the Fmoc-D-Val-OH was incorporated. The final loading as calculated by AAA was 0.106 mmo/g (87% overall yield), the cleavage yield was of the 83%, and 4.7 mg of the title compound were obtained, which represented a 13% overall yield in cyclization, deprotection and purification steps. This product eluted

in HPLC 0.8-1.9 min later than an authentic sample of Kahalalide F [Condition D (t_R 13.3 *vs* 12.5 min), E (t_R 18.8 *vs* 17.4 min), and F(t_R 14.0 *vs* 12.1 min)]. MALDI-TOF-MS, calcd for $C_{75}H_{124}N_{14}O_{16}$, 1,477.9. Found: *m/z* 1,478.7 $[M+H]^+$, 1,500.7 $[M+Na]^+$, 1,516.6 $[M+K]^+$.

The ^{1}H-NMR (500 MHz, d^6-DMSO) spectrum of the compound (Tables II and III) was different from the one obtained for an authentic sample of Kahalalide F. The greatest difference was that the synthetic compound obtained, showed two conformations dued to a cis-trans equilibrium between L-Val-D-Pro residues which was not observed neither in the natural product nor in the isomer obtained in example 7.

Table II (Major, trans isomer)

RESIDUE	N-H	Hα	Hβ	OTHER
(*Z*)-Dhb	9.67 (s)	-	6.33 (q)	1.27 (d, J =7.0 Hz, γ-CH_3)
D-al·lo-Ile 1	8.80 (d)	4.31	1.73	1.32, 0.77 (γ-CH_2, γ-CH_3, δ-CH_3)
L-Phe	8.78 (d, J =5.5 Hz)	4.43	2.93 (m)	7.20 (1 H Ar, m) 7.28 (4H Ar, m)
D-al·lo-Thr	8.58 (d, J =9.0 Hz)	4.53	4.95 (m)	1.07 (d, J =6.5 Hz, γ-CH_3)
L-Val 3	7.97 (d, J =8.0 Hz)	4.34	1.94	0.84 (2 γ-CH_3)
L-Orn	7.77 (d, J =8.5 Hz)	4.47	1.46 (2H)	1.66 (γ-CH_2), 2.72 (bs, δ-CH_2), 7.66 (ε-NH_3^+)

D-al·lo-Ile 2	7.87 (d, J =8.5 Hz)	4.37	1.68	0.75 (δ-CH_3 o d-CH_3 and γ-CH_3)
D-Val 5	7.90	4.22	1.95	0.85 (2 γ-CH_3)
L-Thr	7.90 (d)	4.24	4.02	4.98 (OH), 1.02 (γ-CH_3)
D-Val 2	7.63 (d, J =8.5 Hz)	4.45	2.18	0.77 (γ-CH_3), 0.62 (γ-CH_3)
D-Val 4	7.56 (d, J =9.0 Hz)	4.34	2.02	0.84 (γ-CH_3), 0.79 (γ-CH_3),
L-Val 1	6.75 (d)	3.86	1.39	0.62 (2 γ-CH_3)
D-Pro	-	4.30	2.03, 1.81, 1.73 (β-CH_2, γ-CH_2), 3.73 (1H, m, δ-CH_2), 3.52 (1H, m, δ-CH_2)	
5-MeHex	-	2.08 (1H) 2.15 (1H)	1.48 (b-CH_2, δ-CH), 1.11 (γ-CH_2), 0.82 (2 ε-CH_3)	

Table III (Minor, cis isomer)

RESIDUE	N-H	Hα	Hβ	OTHER
(*Z*)-Dhb	9.63 (s)	-	6.33 (q)	1.28 (d, J =6.5 Hz, γ-CH_3)
D-al·lo-Ile 1	8.76 (d)	4.30	1.71	1.33, 0.76 (γ-CH_2, γ-CH_3, δ-CH_3)

L-Phe	8.78 (d, J =5.5 Hz)	4.43	2.93 (m)	7.20 (1 H Ar, m) 7.28 (4H Ar, m)
D-al·lo-Thr	8.58 (d, J =9.0 Hz)	4.53	4.95 (m)	1.00 (γ-CH_3)
L-Val 3	8.06 (d, J =8.5 Hz)	4.11	1.81	0.71 (γ-CH_3), 0.60 (γ-CH_3)
L-Orn	8.37 (d, J =9.0 Hz)	4.62	1.52 (2H)	1.64 (γ-CH_2), 2.78 (bs, δ-CH_2), 7.66 (ε-NH_3^+)
D-al·lo-Ile 2	8.09 (d, J =9.0 Hz)	4.42	1.64	0.77 (δ-CH_3 or d-CH_3 and γ-CH_3)
D-Val 5	7.90	4.22	1.95	0.85 (2 γ-CH_3)
L-Thr	7.88 (d)	4.23	3.99	4.93 (OH), 1.02 (γ-CH_3)
D-Val 2	7.63 (d, J =8.5 Hz)	4.45	2.18	0.77 (γ-CH_3), 0.62 (γ-CH_3)
D-Val 4	7.49 (d, J =9.0 Hz)	4.34	1.94	0.79 (γ-CH_3), 0.73 (γ-CH_3),
L-Val 1	6.72 (d)	3.84	1.38	0.62 (2 γ-CH_3)
D-Pro	-	4.93	2.06, 1.89, 1.75 (β-CH_2, γ-CH_2), 3.3 (m, δ-CH_2)	
5-MeHex	-	2.08 (1H) 2.15 (1H)	1.48 (β-CH_2, δ-CH), 1.11 (γ-CH_2), 0.82 (2 ε-CH_3)	

Example 15bis

5-MeHex-D-Val-Thr-D-Val-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 15. The product was characterized by HPLC (t_R 17.6 min, Condition B) and MALDI-TOF-MS, calcd for $C_{75}H_{124}N_{14}O_{16}$, 1,477.9. Found: m/z 1,479.3 $[M+H]^+$, 1,501.2 $[M+Na]^+$, 1,517.2 $[M+K]^+$.

Example 16

5-MeHex-D-Val-Thr-D-Val-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val) (*via* incorporation of the dipeptide).

Experimental procedures as described in Examples 8-14 were carried out with the only exception that, as in Example 14, Fmoc-Val-OH was incorporated to D-Pro-peptidyl-resin and then after removal of the Fmoc group, the Fmoc-D-Val-OH was incorporated. The final loading as calculated by AAA was 0.16 mmol/g (79% overall yield), the cleavage yield was of the 78%, and 3.4 mg of the title compound were obtained, which represented a 10% overall yield in cyclization, deprotection and purification steps. The product purified was identical to the one obtained in Example 15.

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Example 17

H-Thr(*t*Bu)-O-TrtCl-resin.

Cl-TrtCl-resin (0.5 g, 1.35 mmol/g) was placed in a 10 mL polypropylene syringe fitted with a polyethylene filter disk. The resin was then washed with CH_2Cl_2 (5 x 0.5 min), and a solution of Fmoc-Thr(*t*Bu)-OH (54 mg, 0.135 mmol, 0.2 equiv) and DIEA (235 mL, 1.35 mmol, 2 equiv) in CH_2Cl_2 (1.25 mL) was added, and the mixture was stirred for 1 h. The reaction was terminated by addition of MeOH (400 mL), after a stirring of 15 min. The Fmoc-Thr(*t*Bu)-O-TrtCl-resin was subjected to the following washings/treatments with CH_2Cl_2 (3 x 0.5 min), DMF (3 x 0.5 min), piperidine-CH_2Cl_2-DMF (1:9.5:9.5, 1 x 10 min), piperidine-DMF (1:4, 1 x 15 min), DMF (5 x 0.5 min), isopropanol (2 x 1 min), DMF (5 x 0.5 min), MeOH (2 x 1 min), and dried over vacuum. The loading as calculated by AAA was 0.15 mmol/g.

Example 18

5-MeHex-Tyr-D-Ser(Gly-H)-Phe-D-Leu-Pro-Thr-OH.

Fmoc-Pro-OH (178 mg, 0.53 mmol, 7 equiv), Fmoc-D-Leu-OH (187 mg, 0.53 mmol, 7 equiv), Fmoc-Phe-OH (204 mg, 0.53 mmol, 7 equiv), Fmoc-D-Ser-OH (free hydroxy group) (173 mg, 0.53 mmol, 7 equiv), Fmoc-Tyr(*t*Bu)-OH (243 mg, 0.53 mmol, 7 equiv), and 5-MeHex-OH (69 mg, 0.53 mmol, 7 equiv) were added sequentially to the above obtained H-Thr(*t*Bu)-O-TrtCl-resin using DIPCDI (82 μL, 0.53 mmol, 7 equiv) and HOBt (81 mg, 0.53 mmol, 7 equiv) in DMF (1.25 mL). In all cases except for D-Ser, after 90 min of coupling, the ninhydrin test was negative. Fmoc-D-Ser-OH (173 mg, 0.53 mmol, 7 equiv) was recoupled with HATU (201 mg, 0.53 mmol, 7 equiv) in the presence of DIEA (184 μL, 1.06 mmol, 14 equiv) in DMF for 90 min. Removal of Fmoc group and washings were carried out as described in General Procedures. Boc-Gly-OH (119 mg, 0.68 mmol, 9 equiv) was coupled

with DIPCDI (105 μL, 0.68 mmol, 9 equiv) in the presence of DMAP (8.3 mg, 68 μmol, 0.9 equiv) for 2.5 h. The coupling was repeated with fresh reagents for 1.5 h. The final loading was 0.10 mmol/g, which represented a 77% synthesis yield. The unprotected peptide was cleaved from the resin (525 mg, 54 μmol) by TFA–H_2O (92:8) for 2 h. The combined filtrates were evaporated to dryness under reduced pressure, H_2O (5 mL) was added, and the solution lyophilized to give 43.0 mg (48.0 μmol, 89% yield) of the title compound with a purity of > 85% as checked by HPLC (Condition A, t_R 18.5 min). ESMS, calcd for $C_{45}H_{65}N_7O_{12}$, 895.5. Found: *m/z* 896.6 $[M+H]^+$.

Example 19

Kahalalide B [5-MeHex-Tyr-*cyclo*(D-Ser-Phe-D-Leu-Pro-Thr-Gly)].

The unprotected peptide (Example 18) (40.5 mg, 45 μmol) was dissolved in DMF (48 mL), and PyBOP (70 mg, 0.135 mmol, 3 equiv) and DIEA (47 mL, 0.271 mmol, 6 equiv) were added. The mixture was allowed to stirr for 2 h, and then the solvent was removed by evaporation under reduced pressure, H_2O (5 mL) was added, and the solution lyophilized. The crude product was purified by medium pressure chromatography (Vydac C_{18} 15-20 μm, 300 Å, 240 x 24 mm), linear gradient from 20% to 60% of acetonitrile (+0.05% TFA) in water (+0.05% TFA) in 5 h (300 mL each solvent), 120 mL/h, detection at 220 nm, to give the title product (8.7 mg, 9.9 μmol, 22% yield). The product was characterized by HPLC (Condition A, t_R 21.6 min) and MALDI-TOF-MS, calcd for $C_{45}H_{63}N_7O_{11}$, 877.5. Found: *m/z* 878.7 $[M+H]^+$, 900.6 $[M+Na]^+$, 916.5 $[M+K]^+$. AAA: Gly 1.02 (1), Thr 0.95 (1), Phe 0.99 (1), Ser 1.00 (1), Pro 1.20 (1), Leu 1.00 (1), Tyr 0.87 (1).

Example 20

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-Etg-Val).

Experimental procedures as decribed in Examples 1-4 and 6-7, starting with 200 mg of resin, were carried out with the only exception that, in Example 4, Fmoc-Thr-OH was replaced by Fmoc-Etg-OH. The product was characterized by HPLC (t_R 16.8 min, Condition B) and MALDI-TOF-MS, calcd for $C_{75}H_{126}N_{14}O_{16}$, 1,479.0. Found: *m/z* 1,480.2 $[M+H]^+$, 1,502.2 $[M+Na]^+$, 1,518.0 $[M+K]^+$.

Example 20b

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-D-Etg-Val).

Experimental procedures as decribed in Examples 1-4 and 6-7, starting with 200 mg of resin, were carried out with the only exception that, in Example 4, Fmoc-Thr-OH was replaced by Fmoc-D-Etg-OH. The product was characterized by HPLC (t_R 17.0 min, Condition B) and MALDI-TOF-MS, calcd for $C_{75}H_{126}N_{14}O_{16}$, 1,479.0. Found: *m/z* 1,501.0 $[M+Na]^+$, 1,517.9 $[M+K]^+$.

Example 20c

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-D-Thr-Val).

Experimental procedures as decribed in Examples 1-4 and 6-7, starting with 200 mg of resin, were carried out with the only exception that, in Example 4, Fmoc-Thr(*t*Bu)-OH was replaced by Fmoc-D-Thr(*t*Bu)-OH.

The product was characterized by HPLC (t_R 19.9 min, Condition B) and MALDI-TOF-MS, calcd for C_{75} H_{126} N_{14} O_{17}, 1,494.9. Found: *m/z* 1,517.4 $[M+Na]^+$, 1,533.4 $[M+K]^+$.

Example 20d

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-allo-Ile-D-Val-Phe-D-*allo*-Thr-Val).

Experimental procedures as decribed in Examples 1-4 and 6-7, starting with 200 mg of resin, were carried out with the only exception that, in Example 4, Fmoc-Thr(*t*Bu)-OH was replaced by Fmoc-D-*allo*-Thr-OH. The product was characterized by HPLC (tR 18.0 min, Condition B) and MALDI-TOF-MS, calcd for C_{75} H_{126} N_{14} O_{17}, 1,494.9. Found: m/z 1,496.6 $[M+H]^+$, 1,518.6 $[M+Na]^+$, 1,534.6 $[M+K]^+$.

Example 20e

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-allo-Ile-cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-D-Phe-Val).

Experimental procedures as decribed in Examples 1--7, starting with 200 mg of resin, were carried out with the only exception that, in Example 4, Fmoc-Thr-OH was replaced by Fmoc-D/L-(b-OH)Phe-OH. The product was characterized by HPLC (t_R 22.2 min, Condition B) and MALDI-TOF-MS, calcd for C_{80} H_{126} N_{14} O_{16}, 1,538.95. Found: *m/z* 1,540.3 $[M+H]^+$, 1,562.4 $[M+Na]^+$, 1,578.3 $[M+K]^+$.

Example 21

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-Dpa-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 2, Fmoc-D-*allo*-Thr-OH was replaced by Fmoc-D-Dpa(Alloc)-OH and that before the incorporation of Alloc-Val-OH, which was incorporated as the rest of protected amino acids, the Alloc group from the Dpa was removed as indicated above. The product was characterized by HPLC and ESMS, calcd for C_{74} H_{123} N_{15} O_{15}, 1,461.9. Found: m/z 1,463.3 $[M+H]^+$.

Example 22

But-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by But-OH. The product was characterized by HPLC (t_R 14.7 min, Condition B) and MALDI-TOF-MS, calcd for C_{72} H_{118} N_{14} O_{16}, 1,435.9. Found: m/z 1,459.6 $[M+Na]^+$, 1,475.6 $[M+K]^+$.

Example 22bis

But-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 22. The product was characterized

by HPLC (t_R 16.0 min, Condition B) and MALDI-TOF-MS, calcd for $C_{72} H_{118} N_{14} O_{16}$, 1,435.9. Found: *m/z* 1,459.5 $[M+Na]^+$, 1,475.5 $[M+K]^+$.

Example 23

3-MeBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 3-MeBut-OH. The product was characterized by HPLC (t_R 15.9 min, Condition B) and MALDI-TOF-MS, calcd for $C_{73} H_{120} N_{14} O_{16}$, 1,449.9. Found: *m/z* 1,473.2 $[M+Na]^+$, 1,489.2 $[M+K]^+$.

Example 23bis

3-MeBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 23. The product was characterized by HPLC (t_R 17.0 min, Condition B) and ESMS, calcd for $C_{73} H_{120} N_{14} O_{16}$, 1,449.9. Found: *m/z* 1,473.3 $[M+Na]^+$, 1,489.4 $[M+K]^+$.

Example 24

3,3-DiMeBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 3,3-DiMeBut-OH. The product was characterized by HPLC (t_R 16.3 min, Condition B) and MALDI-TOF-MS, calcd for C_{74} H_{122} N_{14} O_{16}, 1,463.9. Found: *m/z* 1,487.4 $[M+Na]^+$, 1,503.6 $[M+K]^+$.

Example 24bis

3,3-DiMeBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 24. The product was characterized by HPLC (t_R 17.6 min, Condition B) and MALDI-TOF-MS, calcd for C_{74} H_{122} N_{14} O_{16}, 1,463.9. Found: *m/z* 1,487.3 $[M+Na]^+$, 1,503.3 $[M+K]^+$.

Example 25

4-MePen-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 4-MePen-OH. The product was characterized by HPLC (t_R 16.5 min, Condition B) and MALDI-TOF-MS, calcd for C_{74} H_{122} N_{14} O_{16}, 1,463.9. Found: *m/z* 1,487.7 $[M+Na]^+$, 1,503.6 $[M+K]^+$.

Example 25bis

4-MePen-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 25. The product was characterized by HPLC (t_R 17.8 min, Condition B) and MALDI-TOF-MS, calcd for $C_{74}H_{122}N_{14}O_{16}$, 1,463.9. Found: *m/z* 1,487.8 $[M+Na]^+$, 1,503.6 $[M+K]^+$.

Example 26

Hep-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by Hep-OH. The product was characterized by HPLC (t_R 17.5 min, Condition B) and MALDI-TOF-MS, calcd for $C_{75}H_{124}N_{14}O_{16}$, 1,477.9. Found: *m/z* 1,501.4 $[M+Na]^+$, 1,517.5 $[M+K]^+$.

Example 26bis

Hep-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 26. The product was characterized by HPLC (t_R 18.9 min, Condition B) and MALDI-TOF-MS, calcd for $C_{75}H_{124}N_{14}O_{16}$, 1,477.9. Found: *m/z* 1,501.6 $[M+Na]^+$, 1,517.7 $[M+K]^+$.

Example 27

Pal-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by Pal-OH. The product was characterized by HPLC (t_R 22.1 min, Condition G) and MALDI-TOF-MS, calcd for C_{84} H_{142} N_{14} O_{16}, 1,603.1. Found: m/z 1,626.9 $[M+Na]^+$, 1,642.9 $[M+K]^+$.

Example 27bis

Pal-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 27. The product was characterized by HPLC (t_R 23.2 min, Condition G) and MALDI-TOF-MS, calcd for C_{84} H_{142} N_{14} O_{16}, 1,603.1. Found: m/z 1,626.8 $[M+Na]^+$, 1,642.8 $[M+K]^+$.

Example 27a

4-DiMeABut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 4-DiMeABut-OH. The product was characterized by HPLC (t_R 12.0 min, Condition B) and MALDI-TOF-MS, calcd

for C_{74} H_{123} N_{15} O_{16}, 1,477.9. Found: *m/z* 1,478.6 $[M+H]^+$, 1,500.6 $[M+Na]^+$, 1,516.6 $[M+K]^+$.

Example 27b

2-Hedo-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 2-Hedo-OH. The product was characterized by HPLC (t_R 15.8 min, Condition B) and MALDI-TOF-MS, calcd for C_{74} H_{118} N_{14} O_{16}, 1,458.9. Found: *m/z* 1,460.0 $[M+H]^+$, 1,482.0 $[M+Na]^+$, 1,497.9 $[M+K]^+$.

Example 27c

4-AcBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 4-AcBut-OH. The product was characterized by HPLC (t_R 18.2 min, Condition B) and MALDI-TOF-MS, calcd for C_{74} H_{120} N_{14} O_{18}, 1,492.9. Found: *m/z* 1,493.7 $[M+H]^+$, 1,515.8 $[M+Na]^+$, 1,531.7 $[M+K]^+$.

Example 27d

4-HOBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by 4-HOBut-OH. The product was characterized by HPLC (t_R 16.6 min, Condition B) and MALDI-TOF-MS, calcd for C_{72} H_{118} N_{14} O_{17}, 1,450.9. Found: *m/z* 1,451.6 $[M+H]^+$, 1,473.6 $[M+Na]^+$, 1,489.6 $[M+K]^+$.

Example 27e

Ac-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by HOAc. The product was characterized by HPLC (t_R 17.0 min, Condition B) and MALDI-TOF-MS, calcd for C_{70} H_{114} N_{14} O_{16}, 1,406.9. Found: *m/z* 1,407.8 $[M+H]^+$, 1,429.8 $[M+Na]^+$.

Example 27f

TFA-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (trifluoroacetylion during the cyclization step) of Example 27e. The product was characterized by HPLC (t_R 14.7 min, Condition B) and MALDI-TOF-MS, calcd for C_{70}

H_{111} F_3 N_{14} O_{16}, 1,460.8. Found: *m/z* 1,462.0 [M+H]$^+$, 1,484.1 [M+Na]$^+$, 1,500.0 [M+K]$^+$.

Example 27g

AcButBut-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by AcButBut-OH. The product was characterized by HPLC (t_R 14.1 min, Condition B) and MALDI-TOF-MS, calcd for C_{78} H_{126} N_{14} O_{20}, 1,578.9. Found: *m/z* 1,581.2 [M+H]$^+$, 1,602.2 [M+Na]$^+$, 1,618.2 [M+K]$^+$.

Example 27h

IBut-D-*allo*-Ile-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by Fmoc-D-*allo*-Ile-OH, removal of the Fmoc group and acylation with IBut-OH. The product was characterized by HPLC (t_R 15.3 min, Condition B) and MALDI-TOF-MS, calcd for C_{78} H_{129} N_{15} O_{17}, 1,548.0. Found: *m/z* 1,548.8 [M+H]$^+$, 1,570.8 [M+Na]$^+$, 1,586.8 [M+K]$^+$.

Example 27i

Lit-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by Lit-OH. The product was characterized by HPLC (t_R 13.1 min, Condition H) and MALDI-TOF-MS, calcd for C_{92} H_{150} N_{14} O_{17}, 1,723.1. Found: *m/z* 1,724.6 $[M+H]^+$, 1,746.6 $[M+Na]^+$, 1,761.5 $[M+K]^+$.

Example 27j

TFA-Lit-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (trifluoroacetylion during the cyclization step) of Example 27i. The product was characterized by HPLC (t_R 17.1 min, Condition H) and MALDI-TOF-MS, calcd for C_{94} H_{159} F_3 N_{14} O_{18}, 1,819.1. Found: *m/z* 1,820.6 $[M+H]^+$, 1,842.6 $[M+Na]^+$, 1,858.6 $[M+K]^+$.

Example 27k

TIco-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was replaced by TIco-OH. The product was characterized by

HPLC (t_R 16.8 min, Condition H) and ES-MS, calcd for $C_{92}H_{158}N_{14}O_{16}$, 1,715.2. Found: *m/z* 858.2 $[M+H]^+/2$, 1,171.8 $[M+H]^+$.

Example 28

H-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures as decribed in Examples 1-7, starting with 200 mg of resin, were carried out with the only exceptions that, in Example 3, 5-MeHex was not incorporated and Fmoc-D-Val-OH was replaced by Fmoc-D-Val-OH. The product was characterized by HPLC (t_R 11.6 min, Condition B) and MALDI-TOF-MS, calcd for $C_{68}H_{112}N_{14}O_{15}$, 1,364.8. Found: *m/z* 1,388.3 $[M+Na]^+$, 1,404.3 $[M+K]^+$.

Example 28bis

H-D-Val-Thr-Val-D-Val-D-Pro-Orn-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-Val-Phe-*Z*-Dhb-Val).

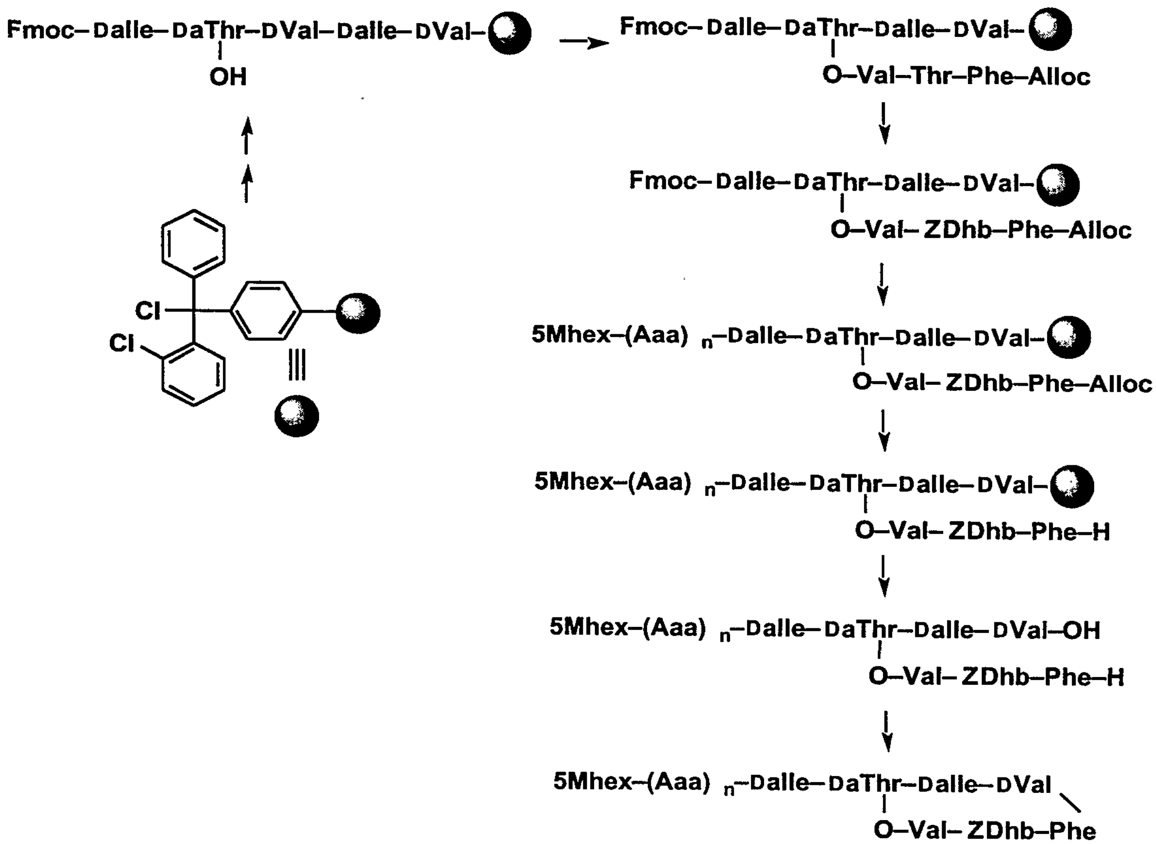
Obtained as a side product during the preparation (racemization during the cyclization step) of Example 28. The product was characterized by HPLC (t_R 12.9 min, Condition B) and MALDI-TOF-MS, calcd for $C_{68}H_{112}N_{14}O_{15}$, 1,364.8. Found: *m/z* 1,388.4 $[M+Na]^+$, 1,404.4 $[M+K]^+$.

Example 29

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures essentially as decribed in Examples 1-7, but according to the scheme 2.

Scheme 2



The synthesis starts with 200 mg of resin and Fmoc-Orn(Boc)-OH was not incorporated. The product was characterized by HPLC (t_R 23.9 min, Condition A) and MALDI-TOF-MS, calcd for C_{70} H_{114} N_{12} O_{15}, 1,362.9. Found: *m/z* 1,386.4 $[M+Na]^+$. 1,402.4 $[M+K]^+$.

Example 29bis

5-MeHex-D-Val-Thr-Val-D-Val-D-Pro-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Obtained as a side product during the preparation (racemization during the cyclization step) of Example 29. The product was characterized by HPLC and MALDI-TOF-MS.

Example 30

5-MeHex-D-Val-Thr-Val-D-Val-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures essentially as decribed in Examples 1-7, but according the scheme 2. The synthesis starts with 200 mg of resin and Fmoc-Orn(Boc)-OH and Fmoc-D-Pro-OH were not incorporated. The product was characterized by HPLC (t_R 20.3 min, Condition B) and MALDI-TOF-MS, calcd for C_{65} H_{107} N_{11} O_{14}, 1,265.8. Found: m/z 1,288.5 $[M+Na]^+$, 1,304.5 $[M+K]^+$.

Example 31

5-MeHex-D-Val-Thr-Val-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures essentially as decribed in Examples 1-7, but according the scheme 2. The synthesis starts with 200 mg of resin and Fmoc-Orn(Boc)-OH, Fmoc-D-Pro-OH, and Fmoc-D-Val-OH were not incorporated. The product was characterized by HPLC (t_R 20.0 min, Condition B) and MALDI-TOF-MS, calcd for C_{60} H_{98} N_{10} O_{13}, 1,166.7. Found: m/z 1,190.9 $[M+Na]^+$, 1,206.9 $[M+K]^+$.

Example 32

5-MeHex-D-Val-Thr-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures essentially as decribed in Examples 1-7, but according the scheme 2. The synthesis starts with 200 mg of resin and Fmoc-Orn(Boc)-OH, Fmoc-D-Pro-OH, Fmoc-D-Val-OH, and Fmoc-Val-OH were not incorporated. The product was characterized by HPLC (t_R 24.6 min, Condition A) and MALDI-TOF-MS, calcd for C_{55} H_{89} N_9 O_{12}, 1,067.7. Found: *m/z* 1,068.7 $[M+H]^+$, 1,090.6 $[M+Na]^+$, 1,106.5$[M+K]^+$.

Example 33

5-MeHex-D-Val-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures essentially as decribed in Examples 1-7, but according the scheme 2. The synthesis starts with 200 mg of resin and Fmoc-Orn(Boc)-OH, Fmoc-D-Pro-OH, Fmoc-D-Val-OH, Fmoc-Val-OH, and Fmoc-Thr(*t*Bu)-OH were not incorporated. The product was characterized by HPLC (t_R 19.8 min, Condition B) and MALDI-TOF-MS, calcd for C_{51} H_{82} N_8 O_{10}, 966.6. Found: *m/z* 990.7 $[M+Na]^+$, 1007.2 $[M+K]^+$.

Example 34

5-MeHex-D-*allo*-Ile-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val).

Experimental procedures essentially as decribed in Examples 1-7, but according the scheme 2. The synthesis starts with 200 mg of resin and Fmoc-Orn(Boc)-OH, Fmoc-D-Pro-OH, Fmoc-D-Val-OH, Fmoc-Val-OH, Fmoc-Thr(*t*Bu)-OH, and Fmoc-D-Val-OH were not incorporated. The product was

characterized by HPLC (t_R 22.0 min, Condition B) and MALDI-TOF-MS, calcd for $C_{46}H_{73}N_7O_9$, 867.6. Found: *m/z* 890.6 [M+Na]$^+$, 906.6 [M+K]$^+$.

Example 35

5-MeHex-*cyclo*(D-*allo*-Thr-D-*allo*-Ile-D-Val-Phe-*Z*-Dhb-Val)

Experimental procedures essentially as decribed in Examples 1-7, but according the scheme 2. The synthesis starts with 200 mg of resin and Fmoc-D-*allo*-Ile-OH, Fmoc-Orn(Boc)-OH, Fmoc-D-Pro-OH, Fmoc-D-Val-OH, Fmoc-Val-OH, Fmoc-Thr(*t*Bu)-OH, and Fmoc-D-Val-OH were not incorporated. The product was characterized by HPLC (t_R 17.1 min, Condition B) and ESMS, calcd for $C_{40}H_{62}N_6O_8$, 754.5 Found: *m/z* 755.7 [M+H]$^+$, 777.7 [M+Na]$^+$, 793.7 [M+K]$^+$.

BIOACTIVITY

The bioactivity of compounds of this invention is demonstrated by the results in the following tables obtained in accordance with the methodology of Berjeron et al., Biochem and Bioph Res. Comm., 1984, 121, 3, 848-854. The cell lines are P388, murine lymphoma; A549, human lung carcinoma; HT-29, human colon carcinoma; MEL-28, human melanoma; DU-145, human prostate carcinoma.

Table

Kahalalide F congeners

Compound	Cycle	Chain**	MW

27j	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: TFA-Lit-	1820
27i	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: lithocholoyl (Lit).	1724
27	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: palmitoyl Palm	1604
27bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal: palmitoyl Palm	1604
27g	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 4-(4-acetoxybutanoyloxy)-butyryl (AcButBut-)	1579
27h	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	IBut-D-allo-Ile-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1548
20e	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-D-Phe-Val) -D-Phe: a,b-didehydro-phenylalanine	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1539
20c	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Thr-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1495
27c	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 4-acetoxibutyryl (4-AcBut)	1493
20	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-L-Etg-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1479
20b	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-D-Etg-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1479
27a	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: N,N-dimethyl-4-aminobutyryl (4-DiMeABut)	1478
(as Scheuer) 15	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-D-Val-L-Val-D-Pro-L-Orn-D-allo-Ile-	1477
15 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-D-Val-L-Val-D-Pro-L-Orn-D-allo-Ile-	1477
26	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: Heptanoyl (Hep)	1477
26 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal: Heptanoyl (Hep)	1477
(Rinehart) 7	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1477
7 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-L-Orn-D-allo-Ile-	1477

24	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 3,3-dimethylbutyryl (3,3-DiMeBut)	1463
24 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal: 3,3-dimethylbutyryl (3,3-DiMeBut)	1463
25	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 3-Methylpentanoyl (4-MePen)	1463
25 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal: 3-Methylpentanoyl (4-MePen)	1463
27f	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: trifluoracetyl (TFA)	1461
27b	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 2,4-hexadienoyl (Hedo)	1459
27d	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 4-hydroxybutyryl	1451
23	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: 3-Methylbutyryl (3-MetBut)	1451
23b	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal: 3-Methylbutyryl (3-MetBut)	1451
22	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: Butyryl (But)	1435
22b	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal:Butyryl	1435
27c	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: acetyl	1407
28	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	Terminal: no fatty acid	1365
28 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	Terminal: no fatty acid	1365
29	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-D-allo-Ile-	1363
29 bis	cyclo(D-allo-Thr-D-allo-Ile-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-Val-D-Pro-D-allo-Ile-	1363
31	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-L-Val-D-allo-Ile-	1167
32	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	5-MeHex-D-Val-L-Thr-D-allo-Ile-	1068
19	L-Phe-D-Leu-L-Pro-_-Thr-Gly-D-Ser	5-MeHex-L-Tyr-	878
34	cyclo(D-allo-Thr-D-allo-Ile-D-	5-MeHex-D-allo-Ile	868

	Val-Phe-Z-Dhb-Val)		
35	cyclo(D-allo-Thr-D-allo-Ile-D-Val-Phe-Z-Dhb-Val)	5-MeHex-	754

** Compounds where the chain is indicated Terminal have the same chain as in compound 7, but with the indicated substitution for the 5-MeHex.

Table
Cytotoxicity of Kahalalide F congeners
IC50 (micromol) values for derivatives

Compound	A549M	DU145M	HT29M	MEL28M	p388M
27j	5,49E-05	5,49E-05	5,49E-05	5,49E-05	2,75E-03
27i	5,80E-04	5,80E-04	2,90E-05	1,45E-04	1,45E-03
27	1,56E-04	3,12E-05	6,23E-05	6,23E-05	3,12E-03
27bis	>3,12E-03	1,56E-03	6,23E-04	6,23E-04	>3,12E-03
27g	>6,33E-04	>6,33E-04	1.58E-03	3,17E-03	>3,17E-03
27h	NA	NA	NA	NA	NA
20e	3,25E-03	6,50E-04	NA	NA	NA
20c	NA	NA	NA	NA	NA
27c	NA	NA	6.70E-04	3.35E-03	NA
20	>3,38E-03	>3,38E-03	>3,38E-03	>3,38E-03	>3,38E-03
20b	>3,38E-03	3,38E-03	3,38E-03	>3,38E-03	>3,38E-03
27a	NA	NA	NA	NA	NA
(as Scheuer) 15	>3,39E-03	3,39E-03	3,39E-03	>3,39E-03	>3,39E-03

15 bis	>3,39E-03	>3,39E-03	>3,39E-03	>3,39E-03	>3,39E-03
26	3,39E-04	3,39E-05	6.77E-04	1,69E-03	3,39E-03
26 bis	>3,39E-03	>3,39E-03	3,39E-04	1,69E-03	>3,39E-03
(Rinehart) 7	3,39E-04	1,69E-04	3,39E-05	3,39E-04	>3,39E-03
7 bis	>3,39E-03	>3,39E-03	>3,39E-03	>3,39E-03	>3,39E-03
24	1,71E-03	3,42E-04	3,42E-04	3,42E-03	>3,42E-03
24 bis	>3,42E-03	>3,42E-03	3,42E-04	>3,42E-03	>3,42E-03
25	3,42E-04	3,42E-04	>3,42E-03	>3,42E-03	>3,42E-03
25 bis	>3,42E-03	>3,42E-03	>3,42E-03	>3,42E-03	>3,42E-03
27f	NA	3,42E-03	>3,42E-03	3.42E-03	3,42E-03
27b	3,43E-03	6,85E-04	3,43E-03	NA	NA
27d	NA	NA	NA	NA	NA
23	>3,45E-03	>3,45E-04	>3,45E-05	>3,45E-04	>3,45E-03
23b	>3,45E-03	>3,45E-03	>3,45E-03	>3,45E-03	>3,45E-03
22	>3,48E-03	1,74E-03	NA	NA	NA
22b	>3,48E-03	>3,48E-03	1,74E-03	>3,48E-03	>3,48E-03
27c	NA	>3,55E-03	>3,55E-03	>3,55E-03	>3,55E-03
28	>3,66E-03	>3,66E-03	>3,66E-03	>3,66E-03	>3,66E-03
28 bis	>3,66E-03	>3,66E-03	NA	NA	NA
29	>3,67E-03	>3,67E-03	>3,67E-03	>3,67E-03	>3,67E-03
29 bis	>3,67E-03	>3,67E-03	>3,67E-03	>3,67E-03	>3,67E-03
31	>4,28E-04	>4,28E-05	>4,28E-03	>4,28E-03	>4,28E-03
32	NA	NA	4,68E-05	4,68E-05	>4,68E-03
19	>5,69E-03	>5,69E-03	NA	NA	NA
34	NA	NA	>5.76E-03	>5.76E-03	>5.76E-03
35	>5,76E-03	>5,76E-03	>5,76E-03	>5,76E-03	>5,76E-03

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Claims

1. A mimic of a natural kahalaide, being a biologically active compound of a structure based on a natural kahalalide, but differing in one or more of the following respects:

at least one amino acid which is not the same as an amino acid present in the parent comopund;

at least one methylene group additional to methylene groups in the side chain acyl group of the parent compound;

at least one methylene group omitted from the side chain acyl group of the parent compound;

at least one substituent added to the side chain acyl group of the parent compound;

at least one substituent omitted from the side chain acyl group of the parent compound.

2. A compound according to claim 1, which is substantially similar in structure to a kahalide selected from kahalalide A, kahalalide B, kahalalide C, kahalalide D, kahalalide E, kahalalide F, kahalalide G, kahalalide H and kahalalide J.

3. A compound according to claim 2, which mimics kahalalide F but differs in the side chain.

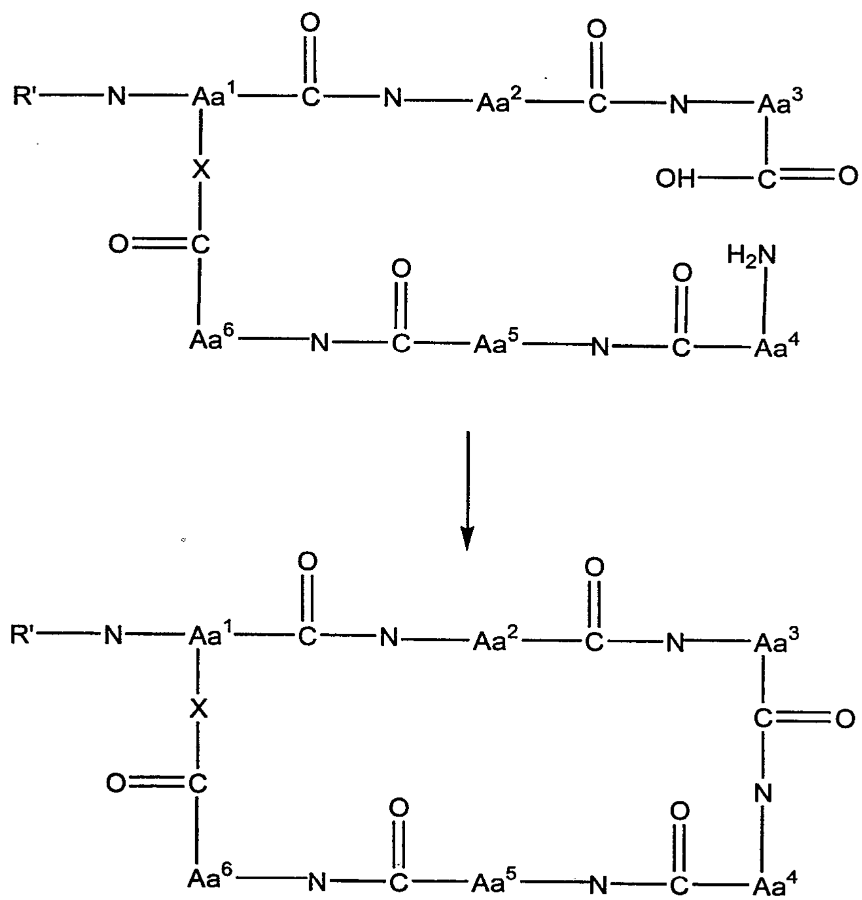
4. A compound according to claim 3, which has at least one additional substituent on the side chain.

5. A compound according to claim 4, which includes at least one halogen substituent.

6. A comopund acording to any of claims 2 to 5, which mimics kahalalide F but differs in a side chain amino acid.

7. A compound acording to any of claims 2 to 5, which mimics kahalalide F but differs in a ring amino acid.

8. A process for preparing a mimic of kahalalide F, which comprises a ring closure according to the following scheme:



where R' is a group R-CO-N- or a precursor therefor, and where one or more of the amino acids may have protecting groups, and where the -COOH of Aa^3 and/or the $-NH_2$ of Aa^4 may optionally be protected, activated or derivatised.

9. A pharmaceutical composition comprising a kahalalide mimic, together with a pharmaceutically acceptable carrier or diluent.

10. A method of treating ancer which includes the administration of an effective amount of a kahalalide mimic or of kahalalide F made by the process of claim 8.

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(81) Designated States (national): AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, BZ, CA, CH, CN, CR, CU, CZ, DE, DK, DM, DZ, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW.

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For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

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(54) **Title:** KAHALALIDE F AND RELATED COMPOUNDS

(57) **Abstract:** A process is provided for preparing kahalalide F and which leads to other kahalalide mimic compounds having useful biological activity.

INTERNATIONAL SEARCH REPORT

Int :ional Application No
PCT/GB 01/00576

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C07K7/08 A61K38/10 A61P35/00

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K A61K A61P

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, WPI Data, PAJ, CHEM ABS Data, BIOSIS, EMBASE, MEDLINE

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	LOPEZ-MACIA A ET AL: "Kahalalide B. Synthesis of a natural cyclodepsipeptide" TETRAHEDRON LETTERS, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 41, no. 50, 9 December 2000 (2000-12-09), pages 9765-9769, XP004223131 ISSN: 0040-4039 the whole document ---	1-10
A	US 6 011 010 A (GRAVALOS DOLORES G ET AL) 4 January 2000 (2000-01-04) ---	
A	EP 0 610 078 A (PHARMA MAR SA) 10 August 1994 (1994-08-10) --- -/--	

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance
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Date of the actual completion of the international search	Date of mailing of the international search report
28 August 2001	07/09/2001

Name and mailing address of the ISA	Authorized officer
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INTERNATIONAL SEARCH REPORT

Int tional Application No
PCT/GB 01/00576

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	GOETZ G ET AL: "The Absolute Stereochemistry of Kahalalide F" TETRAHEDRON, ELSEVIER SCIENCE PUBLISHERS, AMSTERDAM, NL, vol. 55, no. 25, 18 June 1999 (1999-06-18), pages 7739-7746, XP004168551 ISSN: 0040-4020 cited in the application	
A	HAMANN M T ET AL: "Kahalalides: bioactive peptides from a marine mollusk Elysia rufescens and its algal diet Bryopsis sp" JOURNAL OF ORGANIC CHEMISTRY, AMERICAN CHEMICAL SOCIETY. EASTON, US, vol. 61, no. 19, 1996, pages 6594-6600, XP002125310 ISSN: 0022-3263 cited in the application	

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FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.2

Claims Nos.: 1-2

Present claims 1-2 relate to an extremely large number of possible compounds. Support within the meaning of Article 6 PCT and/or disclosure within the meaning of Article 5 PCT is to be found, however, for only a very small proportion of the compounds claimed. In the present case, the claims so lack support, and the application so lacks disclosure, that a meaningful search over the whole of the claimed scope is impossible. Consequently, the search has been carried out for those parts of the claims which appear to be supported and disclosed, namely those parts relating to the compounds which mimic kahalalide F, as further disclosed in claims 3-7, to a process for their preparation and pharmaceutical compositions containing them.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Int ·ional Application No
PCT/GB 01/00576

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